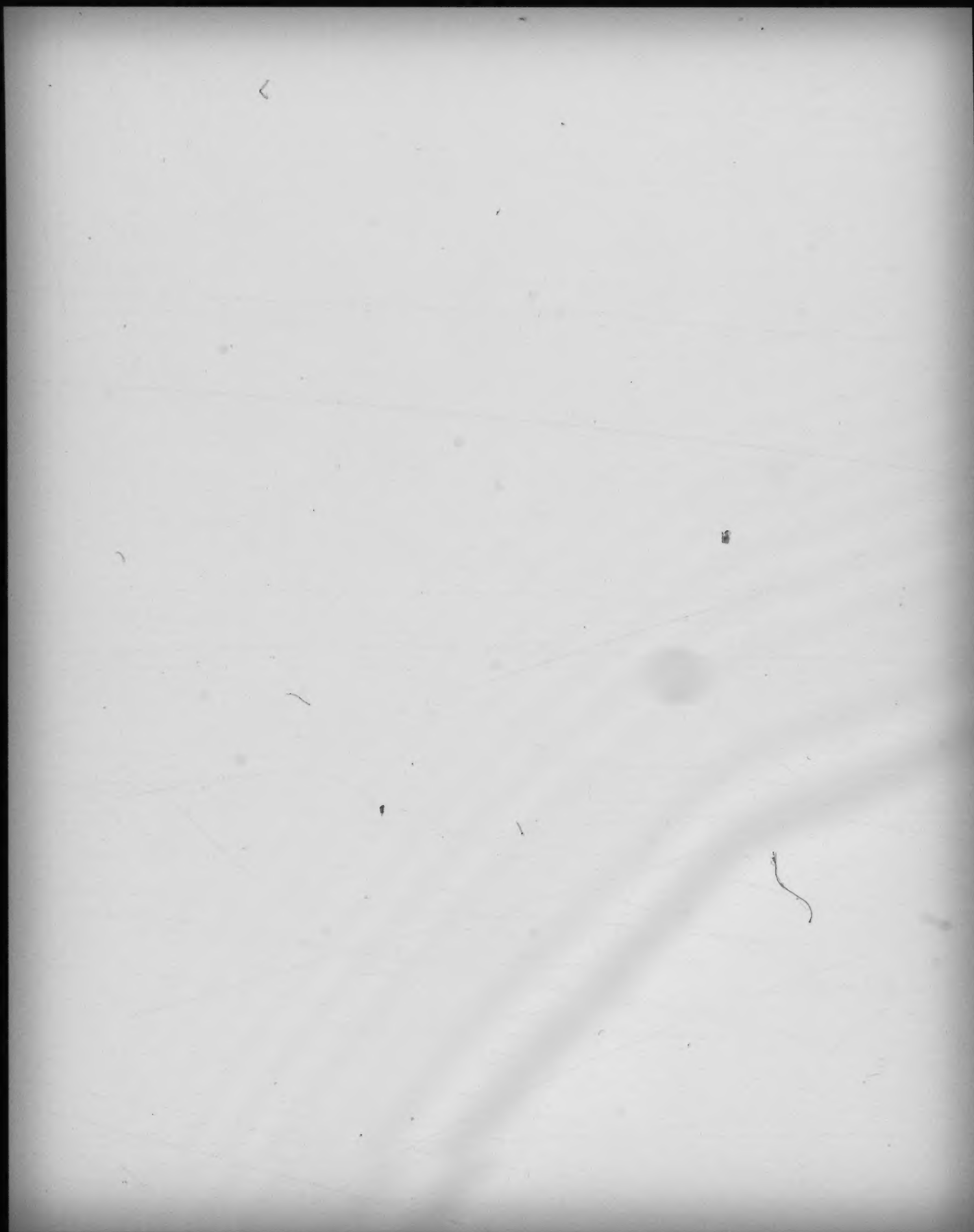


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THE UNIVERSITY OF ALBERTA

**Characterization of Dieters and Effect of an Energy Restriction on
Menstrual Function and Leptin**

by

Sabina Valentine



A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND
RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR A MASTER OF SCIENCE

IN

Nutrition and Metabolism

Department of Agriculture, Food, and Nutrition Science

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THE UNIVERSITY OF ALBERTA
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled *Characterization of Dieters and Effect of an Energy Restriction on Menstrual Function and Leptin* submitted by Sabina Valentine, partial fulfillment of the requirement for a Master of Science in Nutrition and Metabolism

Date Sept 25, 2003
S. Valentine

Abstract

This study hypothesized that decreased energy availability in eumenorrheic obese women results in suppressed reproductive endocrine parameters and decreased leptin, potentially through cortisol. Obese women participated in 7-month weight loss program resulting in 10% weight loss. Leptin and LH were measured for 8 hours during which a GnRH bolus was administered. After weight loss, serum leptin and LH decreased at all time points. Correlations between leptin and hypothalamic-pituitary responsiveness variables were found. Weight loss results in decreased leptin and LH, and decreased responsiveness to a GnRH bolus.

Due to inability of 14/19 subjects to lose weight, an exploratory descriptive follow-up study evaluating characteristics of dieters was performed. Thematic content analysis was used to create categories of processes occurring over time. Subjects who dropped out of the study implemented unsuccessful weight loss strategies. Subjects who remained in the study, but did not lose weight, experienced difficulty managing challenging situations.

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Chapter 1

Literature Review: Part A

1. Introduction

The hypothalamic-pituitary-ovarian (HPO) axis directs reproductive function and is sensitive to changes in the physiological environment. Exercise and nutrition are known to influence ovarian function. The sensitivity of the reproductive axis to nutrition and exercise is demonstrated by the high incidence of reproductive disorders observed in women attempting to lose weight, and in women who are very athletic [1]. The etiology of nutrition- and exercise-related reproductive disorders remains unknown, although a negative energy balance, the stress of exercise and weight loss, and extreme leanness (critical-fat hypothesis) have been suggested as causative factors. The critical-fat hypothesis has been challenged [2], however, this theory continues to receive widespread support from the general public.

Identification of the adipocyte-derived *ob* gene product, leptin, has provided a possible link between fat and fertility. The suggestion that leptin may be involved in regulating reproductive function during periods where energy is restricted arises from indirect evidence showing that leptin may affect reproductive function in humans and rodents [3, 4]. Leptin may respond to metabolic signals occurring with an energy deficit, and then may, itself, act as a peripheral signal that mediates reproductive endocrine parameters. Alternatively, leptin may act as a mediator of a peripheral signal that affects reproductive function, which may then affect reproductive endocrine parameters. This literature review will focus on the peripheral signal involving leptin that communicates information about energy availability to the reproductive axis at the central level.

2. Predisposing Factors for Menstrual Disorders

The most commonly reported reproductive disorder that occurs as a result of a chronic energy deficiency is secondary amenorrhea, or cessation of menstrual function for 3 or more consecutive cycles [1]. The prevalence of secondary amenorrhea in the general population is 1-5% [1], up to 66% in athletes [5], and 100% in women with anorexia nervosa [6]. Amenorrhea is often characterized by suppressed LH hormone pulse frequency, decreased estrogen and progesterone levels, abnormal follicular development, and suppressed ovarian function [7]. These adaptations are associated with transient infertility, premature loss of bone, increased breast cancer risk, and increased cardiovascular disease risk [1]. The main cause of secondary amenorrhea is linked to defective GnRH pulse release [8]. Yet, the mechanism by which GnRH pulse release is impaired remains unclear.

A number of predisposing factors are suggested as probable causes. These include body fatness, energy intake, intensity and duration of exercise training, psychological and physical stress, predisposition to menstrual irregularities (genetic), and delayed menarche [1]. These factors are not mutually exclusive and may act in combination to cause menstrual disorders.

2.1. Critical Fat Hypothesis

The critical fat hypothesis states that delayed menarche and menstrual dysfunction occur when body fat drops below a critical level of 17% and 22%, respectively [9]. This is supported by observations that amenorrheic athletes are lighter and leaner [10], that menstrual irregularities are more common in sports emphasizing low body fat [11], and

that women with a high percentage of body fat are resistant to inhibition of gonadotropin secretion during long periods of fasting [12].

The critical fat hypothesis attempts to explain menstrual disturbances occurring with an energy deficit by evaluating the resulting changes in body fat. Research investigating this hypothesis found no clear relationship between menstrual irregularities and body fat levels [2]. Inaccurate assessment of body fat using a height-weight equation, and the simplistic approach of the hypothesis have provided evidence against the critical fat hypothesis as a mechanism by which menstrual dysfunction occurs with an energy deficit.

2.2. Metabolic Hypothesis

Mechanisms that link reproduction, net energy intake, and fuel partitioning are controlled by a sensory system that regulates metabolic signals [13]. Changes in intracellular metabolic fuel availability and oxidation, rather than changes in body fat, generate these signals [13]. The metabolic system allocates energy to varying functions, depending on the amount of energy available for those functions. Thus, when an energy drain is created by reduced energy intake with or without an increased energy output, a negative energy balance (energy intake is less than energy expenditure) occurs and some physiological functions are maintained at the expense of others. The body prioritizes energy needs, sustaining energy for thermoregulation, cellular maintenance, and locomotion, at the expense of energy required for reproduction, growth, and adipose tissue storage [14]. The dependence of reproduction on energy availability (defined as dietary energy intake minus exercise energy expenditure [15]) in mammals, including

humans, led to the proposal that reproductive dysfunction accompanied by an energy deficit is due to inadequate energy intake needed to meet the demands for energy expenditure [7].

Evidence that a negative energy balance, resulting from increased energy expenditure or decreased dietary intake, plays a causal role in the development of exercise-induced disturbances in menstrual function comes from several studies. Various human and animal studies show that a negative energy balance, occurring as a result of short- [16-18] or long-term [14, 19] decreases in food intake, is tightly associated with suppression of reproductive function. Decreased energy availability created by diet or exercise results in a change in LH pulsatility [20, 21], while a subsequent increase in energy availability results in a resumption of LH pulsatility [21].

The energy deficit created by exercise, rather than the exercise stress itself, is capable of impairing GnRH release [7]. Decreases in LH pulse frequency occur in subjects who exercise and are fed fewer calories than are necessary to maintain body weight as compared to subjects who exercise while being fed enough calories to maintain weight [7, 22]. Amenorrhea induced by a long-term energy deficit created by a 7-24 month exercise program in female adult monkeys (where energy intake remained constant during exercise training), is reversed when supplemental calories are provided while monkeys maintain their daily training [23]. These results suggest that the energy cost associated with exercise, rather than other exercise-associated factors, such as physical or psychological stress, play a causal role in the development of exercise-induced amenorrhea.

There may be a threshold level of energy availability, below which reproductive function is compromised. Loucks and Thuma [15] recently found that LH pulsatility, measured in the early follicular phase, is unaffected by an energy availability of 30 kcal/kg LBM d (where subjects ingested 45 kcal/kg LBM d and expended 15 kcal/kg LBM d) in young women. Below this threshold, LH pulse frequency decreases, while LH pulse amplitude increases [15]. Disruption of LH pulsatility below a threshold energy availability of 30 kcal/kg LBM d (about 1330 kcal/d for 45 kg LBM subjects) suggests that LH pulsatility is not linearly proportional to energy availability [15].

With an energy deficit, metabolic compensation occurs to conserve energy by shifting metabolic fuels away from reproductive function. Energy conservation in athletes with menstrual disorders is suggested by some studies [24-26], but not all [27, 28], who report that amenorrheic athletes consume fewer calories than eumenorrheic athletes when they are matched for training level, height, and body weight. Athletes with exercise-induced menstrual disturbances have lower resting metabolic rates than either eumenorrheic athletes or sedentary women [20, 29], while others find no differences in the metabolic rates of amenorrheic subjects as compared to eumenorrheic athletes or sedentary women [28]. The decrease in triiodothyronine (T3) levels in amenorrheic athletes as compared to cyclic athletes and sedentary women [8, 30], and the decrease in T3 during the induction of exercise-associated amenorrhea in primates [23] may also be adaptive responses to an energy deficient state to conserve energy and alter the allocation of energy resources.

With an energy deficit, a metabolic factor, which changes in parallel with changes in circulating T3 concentrations and metabolic rate, may provide the critical cue linking

reproductive function to metabolic status. A number of metabolic substrates and hormones are proposed to play such a role, including decreased leptin [8, 31] decreased plasma glucose [32, 33], increased human growth hormone [32, 33], and cortisol [32, 33], decreased insulin [32, 33], and decreased IGF-I [33]. These metabolic and hormonal changes may represent metabolic responses that reallocate metabolic fuels away from the reproductive axis toward other important life-sustaining metabolic functions to conserve energy during periods of low energy availability [34].

Although the energy availability hypothesis represents a plausible mechanism by which an energy deficit disrupts reproductive function, the mechanistic underpinnings linking decreased energy availability to reproductive disorders are not known. Additionally, there are a limited number of studies investigating the effect of a chronic energy deficit on reproductive function. Many studies linking reproductive function and energy availability are correlational in nature, and are thereby unable to establish a causal relationship between these states

2.3. Stress

Although other hormones and metabolites respond to increases in stress, extensive investigation of the HPA axis in relation to stress has been completed due to the impact that increased activity of the HPA axis may have on reproductive function, and other physiological variables. The high cortisol levels observed in amenorrheic athletes [14] and in women with anorexia nervosa (who are amenorrheic by definition; [7]) [35] led to suggestions that excessive exercise by itself or in combination with a low energy intake may act as chronic stressors that activate the hypothalamic-pituitary-adrenal (HPA) axis.

Corticotropin-releasing hormone (CRH) reduces GnRH secretion directly from the arcuate neurons of the hypothalamus or indirectly by increasing activity of opioids and serotonin [35].

Activation of the HPA axis, occurring with an energy deficit or due to the physical stress of exercise, may suppress reproductive function. The HPA axis suppresses GnRH release in underweight women with anorexia nervosa, who chronically restrict their energy intake and experience a great deal of psychological stress [36]. Additionally, the physical stress of acute exercise may reduce adrenal sensitivity and increase cortisol levels, which is associated with impairment of menstrual function [37].

In contrast, various studies have found that diet- and exercise-induced changes in LH pulsatility are not influenced by HPA hormones. Diet-induced changes in LH pulsatility were not influenced by cortisol, but were reversed upon refeeding in rhesus monkeys [38]. A 10% increase in cortisol levels occurred as a result of four days of extreme exercise training, and did not affect LH pulsatility when the energy deficit created by energy expenditure was matched by an equally large increase in energy intake [7]. This indicates that stimulation of the HPA axis may not have a negative effect on the HPO axis when the subjects are in a state of energy balance [7]. Varying degrees of HPA involvement on the suppression of reproductive function may be due to the differing study designs, subject types, and the degree/duration of energy deficits utilized. It is also possible that other hormones, such as insulin and leptin, are involved in the regulation of reproductive function with an energy deficit.

3. Leptin: Actions and Regulation

Leptin is a 16-kDa protein secreted predominantly from adipocytes, which may play a key role in serving as a metabolic cue regarding energy balance and metabolic status to control reproductive function [14, 39, 40]. The expression and secretion of leptin is proportional to the amount of body fat mass and the adipocyte size [41]. Leptin forms an integral part of a complex feedback loop in regulating energy homeostasis by providing information about food intake, energy expenditure, and whole body energy balance from adipose tissue to the central nervous system [41]. Leptin has been suggested to decrease energy intake and increase energy expenditure by inhibiting neuropeptide Y (NPY) [42]. The role of leptin in regulating energy balance has led to the suggestion that leptin may provide a link between nutrition, adipose tissue and reproduction. Leptin may act as a peripheral signal that reports energy status to the hypothalamic regulators of reproduction [14].

Plasma leptin has a distinct and clear circadian rhythm where the zenith occurs near midnight, and the nadir occurs in the early to mid-morning [43-46]. Research has shown that the 24-hour leptin rhythm is responsive to meal timing as seen by a decrease in leptin levels for an additional 6-7 hours with a 6.5 hour delay in breakfast, and a subsequent slow rise in leptin with each progressive meal [47]. Leptin receptors share homology with the class I cytokine receptor family [44]. There are a number of different splice variants of the leptin receptor. The long form of the leptin receptor is expressed primarily in the hypothalamus and activates the JAK-STAT (Janus kinase-signal transducers and activators of transcription) pathway [48].

Leptin is regulated by a number of hormones and neurotransmitters. Increased adipocyte size, estrogenic hormones, insulin/glucose/hexosamines, glucocorticoids, growth hormone/insulin-like growth factor-1 [49], and acutely elevated tumor necrosis factor-alpha (TNF- α) are all suggested to stimulate leptin release from adipose tissue [50]. Androgenic hormones, peroxisome proliferator-activated receptor-gamma (PPAR- γ) agonists, catecholamines, and chronically elevated TNF- α may all inhibit leptin release from adipose tissue [50]. The sympathetic nervous system, which innervates adipocytes, is also a main physiological regulator of leptin production and participates in a negative feedback loop to adipose tissue in the production of leptin where it inhibits leptin synthesis through the stimulation of beta-3-adrenergic receptors in rodents [51]. As explained in greater detail below, serum leptin levels are also altered by metabolic and hormonal changes occurring with changes in energy intake and energy expenditure.

4. Leptin and Adiposity

In humans and rodents, *ob* mRNA and leptin levels are positively correlated with the amount of adipose tissue, although there is a wide interindividual variation in leptin levels at any given level of body fat [52]. This suggests that people with higher levels of body fat have higher leptin levels, such as in women in comparison to men, and in obese persons in comparison to lean persons. Reasons for the higher leptin levels in relation to the amount of fat mass for women and for obese persons are discussed below.

4.1. Gender Differences in Leptin

Leptin levels have been suggested to be secreted in direct proportion to body fat values [52]. However, even after correcting for body weight and fat mass, women have higher serum leptin levels than men [53, 54]. This disproportionately high leptin level in relation to fat mass in women is due to a number of factors. First, the pulse amplitude, but not the pulse frequency, of leptin secretion from adipose tissue is twofold to threefold higher in females than in males [55]. Second, fat mass is increased in females, and there is a differential fat distribution with an increased subcutaneous/visceral fat ratio in women than men. Leptin mRNA expression has been suggested to be higher in subcutaneous than visceral fat depots [56, 57]. Third, women have higher total serum leptin levels but lower leptin-binding protein levels than men, signifying higher free leptin levels [58]. Finally, female adipose tissue may be more sensitive to hormones or other substances that stimulate leptin production. It is known that sex steroids such as estrogen increase leptin levels [59], whereas androgens decrease leptin levels [60]. Thus, a number of factors may account for the increased levels of leptin in women, as compared to men with the same body fat value.

4.2. Leptin and Obesity

The role of leptin in regulating energy balance has motivated many researchers to examine the role of leptin in obesity. Genetic obese mouse strains, such the *ob/ob* and *db/db* mice have provided models in which to study the role that leptin may have in human obesity. The *ob/ob* mice are unable to produce leptin, while *db/db* mice have a defective leptin receptor and are considered to be 'leptin resistant' [49]. Both strains

display a similar phenotype, with severe and early-onset obesity, extreme insulin resistance, hyperphagia, reduced energy expenditure and infertility in both sexes [61, 62].

Human obesity appears to more closely resemble the leptin-resistant *db/db* mouse in that circulating leptin levels reflect adiposity in obese humans [63]. Despite the high leptin levels, excessive adipose tissue is maintained in obesity, leading to the suggestion of a form of leptin resistance that cannot be overcome by the high plasma levels of leptin. This concept is also supported by the observation of a reduced cerebrospinal fluid/plasma ratio of leptin content [64], suggesting a defective transport of the hormone through the blood-brain barrier. Another alteration present in obese persons is that the bound/free ratio of plasma leptin is reversed, compared to normal weight subjects. In obese subjects, free leptin accounts for 70 - 80% of total plasma leptin, while in lean subjects free leptin is only about 10-15% of total [63]. This change might be due to a defective parallel increase of leptin binding protein in obese people and further suggests the existence of leptin resistance in human obesity [63].

High leptin levels seen in obese women may contribute to anovulation occurring in obese women [40]. This is consistent with research showing that the induction of hyperleptinemia uncoupled from obesity-induced leptin resistance may cause late onset reproductive failure in transgenic skinny mice overexpressing leptin [65]. These findings are supported by the direct inhibitory action of high leptin levels on ovarian steroidogenesis leading to ineffective follicular maturation. Additionally, the observation that obese girls reach menarche at an earlier age compared to normal weight girls [66] suggests that high leptin levels may contribute to reproductive dysfunction in obese women not only at the ovarian level, but also at the central level of the hypothalamic-

pituitary-ovarian axis, where increasing leptin levels may have an effect on stimulating early menarche [40].

However, many other factors govern the reproductive function of an obese woman. For example, hyperinsulinaemia and insulin resistance are widely accepted to be involved in the underlying mechanisms linking obesity to multiple metabolic abnormalities and to alterations on steroidogenesis [67]. Insulin exerts a variety of direct actions on steroidogenesis in humans, stimulates ovarian estrogen, androgen, and progesterone secretion *in vitro* [67]. Although the mechanism by which the excess adipose tissue is linked with reproductive dysfunction is controversial, high insulin levels have been suggested to play a primary role in the oligo-ovulation, anovulation, and subfertility that are present in obese females [68]. This suggests that normalization of insulin levels and resistance would be associated with normal reproductive function. The overall influence of reproductive dysfunction on reproductive health in obese women is minimal as the incidence of reproductive dysfunction in obese women is low, and less than the incidence of reproductive dysfunction in normal weight women [69].

5. Leptin Levels Decrease with an Energy Deficit

In general, leptin expression and levels decrease in response to a short- and long-term energy deficit induced by a low energy intake or high energy expenditure, in both humans and rodents [41, 52]. Low leptin levels act as a signal that coordinates the response to reduced energy stores by directing energy towards food-seeking behaviour, conserving energy by reducing sympathetic outflow to adipose tissue, reducing secretion of thyrotropin and growth hormones, and switching off reproductive function by reducing

gonadotropin release [49]. These decreases in plasma leptin levels were suggested to be both dependent [41, 70, 71] and independent of fat mass [41]. Disproportionate decreases in leptin in relation to decreases in fat mass were suggested to be due to changes in body fat distribution [41], or changes in metabolic variables.

5.1. Decrease in Fat Mass with an Energy Deficit: Leptin Response

A 10-15% loss of weight in human subjects results in a 22-53% decrease in leptin [41, 52, 70, 71], which is suggested to be both dependent [41, 70, 71] and independent of fat mass [41]. Studies that have found that decreases in leptin with an energy deficit occur in direct proportion to decreases in fat mass [41, 70-72] are consistent with the finding that leptin expression is positively correlated with adipocyte hypertrophy and hyperplasia [73]. Disproportionate changes in leptin relative to fat mass with weight loss may be due to loss of more or less subcutaneous fat compared to omental fat, as more leptin is produced by subcutaneous versus omental fat [41].

Alternatively, the decrease in leptin concentrations beyond the anticipated value for the reduction in body fat [41, 74 - 77], suggest that the decrease in leptin operates independently of the available adipose tissue triglyceride stores [78]. An energy deficit-induced decline in leptin may occur as a result of metabolic changes such as a change in fuel flux [75], an increase in free fatty acids (FFAs; [79, 80] and ketones [78], a decrease in plasma glucose/insulin levels [81], and changes in glucose metabolism [82]. These metabolic changes may have additive effects, thereby resulting in a decrease in leptin beyond that expected for decreases in fat mass.

5.2. Leptin and Exercise

Exercise may affect leptin levels and leptin actions through changes in fuel flux, systemic hormone levels, and energy expenditure. Research characterizing the response of leptin to acute exercise is controversial. Cross-sectional studies and acute exercise studies show that the changes in leptin are not independent of changes in fat mass [83-85], that acute exercise had little or no effect on plasma leptin [86-90], or that changes in plasma leptin may occur 2-48 hours after the exercise bout [91, 92]. Similarly, the effect of chronic exercise on leptin is very controversial where some studies show changes in leptin that are dependent on fat mass [90, 93] and others show changes in leptin that are independent of fat mass [94, 95]. Sampling of only a single fasting plasma leptin level and the lack of careful control of energy balance may account for the inconsistent results found between studies investigating the effects of acute and chronic exercise on plasma leptin levels.

A well controlled energy balance study utilizing a 24-hour assessment of plasma leptin levels found that the diurnal rhythm and the amplitude of leptin are suppressed when energy availability is decreased by diet or exercise when compared to the energy balanced condition (which also involved exercise; [96]). Therefore, leptin does not respond to individual changes in energy intake, or expenditure, but responds to energy availability, that is, the difference between energy intake and energy expenditure.

6. Leptin and Reproductive Function

The dependency of the HPO axis function on leptin under conditions of energy equilibrium in mice suggests that reproductive dysfunction during periods of energy

imbalance, such as amenorrhea, may also be linked to leptin. With an energy deficit, leptin may function as a 'barometer' of the body's energy status that sends signals to the brain, to affect the release of GnRH or gonadotropins.

General evidence that leptin is related to reproductive function includes research showing that exogenous leptin administration in rodents reverses the effects of food deficit or deprivation on aspects of reproduction, including the onset of puberty [97-101], the length of the estrous cycle [3, 102], the length of lactational diestrus [103], gonadotropin and gonadal steroid levels [104, 105], and pulsatile LH secretion [106, 107] in mice, rats, hamsters, and nonhuman primates. The level of the reproductive axis that is affected by leptin is not known. Leptin may modulate reproductive hormones at the central level as seen by increments of LH during weight gain in patients with secondary amenorrhea due to acute anorexia nervosa, that parallel increments of leptin [33], and the strong synchronization between LH and leptin pulses observed in blood collected every 10 minutes for 6 hours in normally cycling women. Alternatively, leptin may modulate reproductive hormones at the ovarian level [48, 49].

Leptin may regulate reproductive endocrine parameters directly or indirectly by altering the secretion or expression of hormones, or by altering the responsiveness of the pituitary gland to GnRH, rather than by directly altering the secretion or expression of hormones. Changes in pituitary responsiveness occurring as a result of decreases in leptin, mediate the decrease in LH pulse frequency with a 48-hour fast in adult female rats [4]. Leptin administration, *in vitro*, enhances the responsiveness of the anterior pituitary to GnRH stimuli and potentiates GnRH release from hypothalamic explants [104]. Alternatively, changes in leptin binding, distribution of leptin receptors, or leptin

secretion within tissues such as brain, liver, or muscle may result in changes in reproductive function [108-110]. From these tissues, afferent projections to the CNS areas that control the GnRH pulse generator and food intake may be involved in regulating leptin [13]. Other researchers suggest that the reproductive effects of leptin in the absence of changes in plasma leptin levels may be due to changes in the binding of circulating leptin to a high-affinity binding protein [111].

It is also possible that leptin does not have a role in regulating reproductive function at all. Normal estrous cycles can occur in hamsters when leptin concentrations are not significantly above those of fasted anestrus hamsters [112]. Thus, leptin levels above fasting concentrations do not appear to be required for normal estrous cyclicity.

7. Direct Effect of Leptin on Reproductive Endocrine Parameters.

The decreases in peripheral leptin occurring with an energy deficit may directly regulate GnRH secretion, or the secretion of gonadotropin hormones, at the central level by crossing the blood brain barrier [113]. This results in a decrease in GnRH release, synthesis, or secretion, as leptin has been shown to stimulate GnRH [114]. Research suggests that leptin may exert direct action on the GnRH neurons by modifying its inherent pulsatility or capacity to express GnRH [114]. This is consistent with the report that leptin receptors are expressed in mouse immortalized GnRH neurons [115] and that these receptors seem to be functional in modulating GnRH secretion [39]. However, little coexpression of the leptin receptor (Ob-R) in conjunction with GnRH at either the protein or mRNA level has been detected in either monkey or rat models. Finn et al [106] did not identify any distinct clusters of silver grains over GnRH neurons that would

indicate clear expression of Ob-R mRNA by these cells. Thus, few, if any, GnRH neurons are found to express Ob-R protein in the rat.

Other evidence suggests that leptin does not directly affect expression and secretion of GnRH and gonadotropins. Leptin treatment did not prevent changes in LH secretion observed with a short-term energy deficit in male adult [116] and pubertal monkeys [117], as well as fasted male mice [105]. Short- and long-term reductions in energy intake in normal weight women and infertile obese women respectively, result in decreases in leptin that are unrelated to changes in LH [118, 119]. Leptin levels are not significantly different in women with hypothalamic amenorrhea and women who are normally cycling, despite differences in LH pulse frequency [120, 121]. Amenorrhea in athletes is associated with a loss of the diurnal rhythm of leptin, even though leptin levels were similar to normally cycling women [8]. Although the data are often correlational in nature, it is possible that leptin may affect reproductive function through a loss of diurnal rhythm and change of pulsatility parameters rather than through a decreased expression and secretion [8]. Discrepancies between studies about the effects of leptin on LH secretion may be due to the multiple endocrine abnormalities in the subjects studied, to the pulsatile nature of LH and leptin secretion and the fact that, in many studies, measurements are made on single samples.

Leptin may act as a metabolic signal to the reproductive system through direct action on the gonads [122] as leptin receptors are reported in human granulosa, theca, and interstitial cells [122]. Various studies have shown that leptin has inhibitory [123] and stimulatory effects [124] on ovarian steroid output, depending on the model and the subject group used to assess ovarian steroid output. Other hormones, such as insulin,

may also be important signals in the control of ovulatory cycles [125, 126]. Further studies are needed to clarify if leptin and insulin play a role in communicating the metabolic status to the reproductive system by affecting ovarian hormones.

Although it is possible that decreases in leptin occurring with an energy deficit directly limit reproductive function by acting at the central or ovarian levels of the reproductive axis, the complexity of the hormonal and metabolic response to an energy deficit suggests that leptin most likely regulates reproductive function through the interaction with a peripheral signal. The regulation of reproductive function by leptin through the interaction with a peripheral signal is consistent with the location of leptin receptor binding, leptin receptor immunoreactivity, or leptin receptor mRNA (primarily mRNA for the short isoform of the receptor) in organs outside of the CNS such as adipose tissue, pancreas, liver, kidney, lung, adrenal medulla, and skeletal muscle [100, 110, 122, 123]. Leptin may affect fuel oxidation in these tissues and these tissues, in turn, may generate signals carried by afferent projections to areas that control reproduction. Thus, the peripheral mechanism by which leptin communicates information about energy availability to the central level of the reproductive axis, is most likely indirect (Figure 1.1).

8. Indirect Effect of Leptin on Reproductive Endocrine Parameters

Leptin may regulate reproductive endocrine parameters at the peripheral level through the effect of leptin on metabolites or activity of the HPA axis (Figure 1.1). The link between leptin, metabolites, and reproductive endocrine parameters evolved from the metabolic hypothesis that leptin may affect reproductive endocrine parameters by acting

Figure 1.1: Summary of the peripheral mechanism by which leptin may affect reproductive function with an energy deficit.

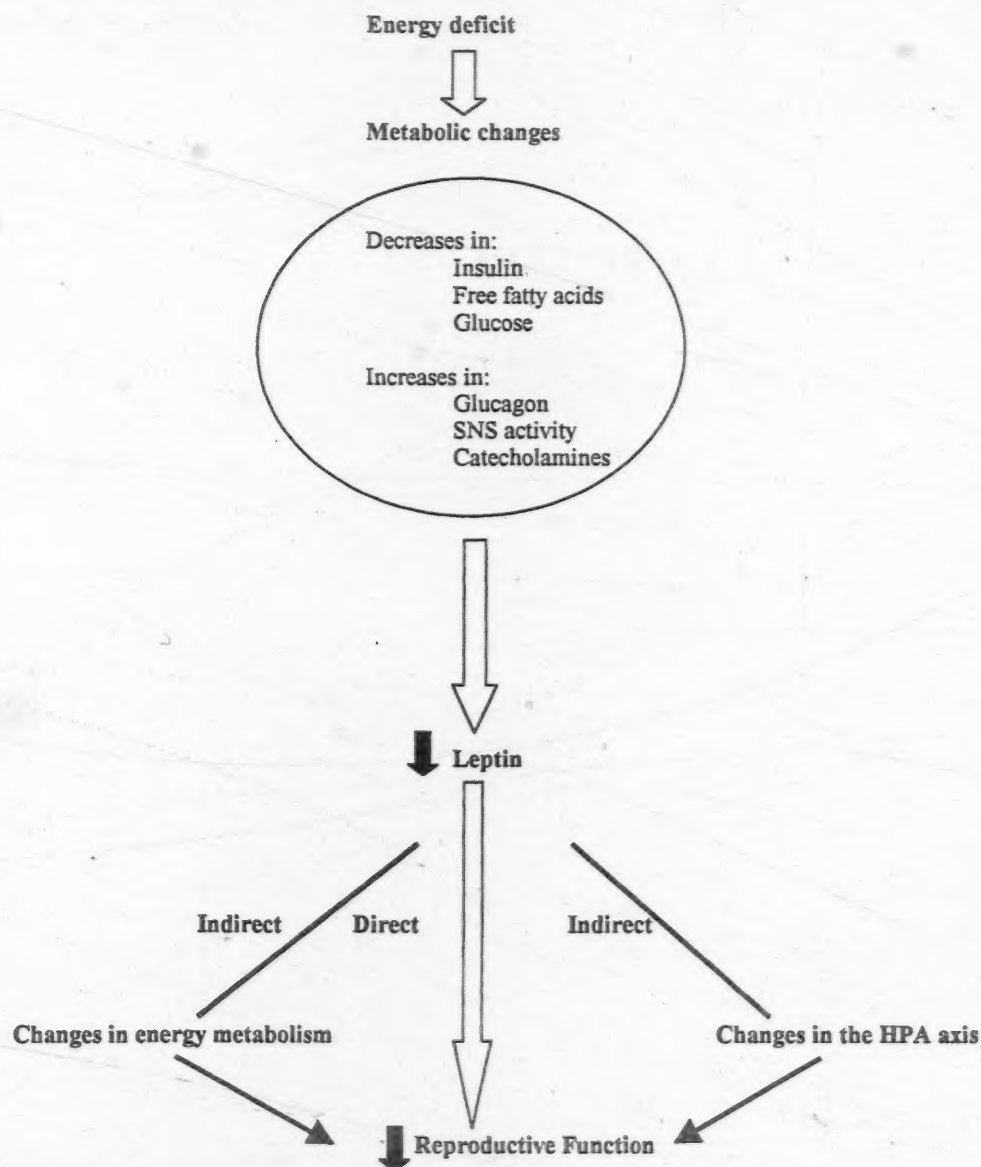


Figure 1.1 description: An energy deficit causes various metabolic changes, which may decrease leptin levels. From the periphery, leptin may affect reproductive function at the central level either directly or indirectly through changes in HPA axis or energy metabolism. Whether indirect or direct, leptin may signal the reproductive axis of decreased availability of oxidizable fuels available for reproductive function.

as a mediator of metabolic signals occurring with an energy deficit [13]. The link between the HPA axis, leptin and reproductive function arose from the observations that increases in cortisol that can occur with an energy deficit and inhibit reproductive function [97], may be related to changes in leptin.

Leptin was initially suggested to affect reproductive function through a link with adiposity, proposed by studies in *ob/ob* mice (obese mice who are unable to produce a functional form of leptin). According to the lipostatic theory, plasma leptin levels provide the critical information about the amount of body fat available to sustain reproductive function [44, 101, 127] where low and high levels of body fat would inhibit and promote reproductive function respectively [128]. However, the link between leptin and reproductive function through body fat stores is questionable, as plasma leptin levels have been found to reflect short-term changes in fuel availability more accurately than they reflect body fat content [78, 79, 129]. Large decreases in leptin without a change in estrous cyclicity in fasted, fat hamsters, supports the notion that leptin does not act as an adipostatic signal that reflects body fat content and regulates reproductive function [112]. Thus, leptin may be classified more accurately as a mediator of metabolic signals that influence reproduction, rather than an "adipostatic" signal [13].

The metabolic hypothesis suggests that metabolic signals are generated by changes in intracellular metabolic fuel availability and oxidation, and that leptin may affect reproductive endocrine parameters by acting as a mediator of these metabolic signals [13]. Effects of leptin administration that involve the area postrema, which assimilates signals from leptin and fuel availability [13], are consistent with the belief that exogenous leptin treatment affects reproduction through its effects on fuel oxidation.

These results suggest that inhibition of gonadotropin secretion may be partly attributable to low plasma levels of leptin [106]. Furthermore, luteal phase deficiencies in exercising women [130], amenorrhea in female athletes [8], and amenorrhea occurring in exercising monkeys all suggested to occur as a result of decreases in energy availability, are associated with decreases in plasma leptin levels [23, 130]. These researchers suggested that it is possible that leptin may act as a communication link between adipocytes and the hypothalamus, supplying the hypothalamus with peripheral cues concerning energy balance and metabolic status to control reproductive function [14, 39, 40].

The levels of plasma leptin may be an indicator of the fuel available to maintain reproductive function, where a threshold level of leptin may be associated with reproductive dysfunction. Although it is an associated finding, it is suggested that when leptin falls below a certain "critical threshold level" of 1.85 ng/ml [131] in an energy-deprived state, changes in reproductive hormone parameters as well as impairment in follicular development that characterize individuals in a starvation period, are invoked to conserve metabolic fuel [132].

This research shows that decreases in energetic fuel availability, which may be communicated through metabolic changes occurring with an energy deficit, may be involved in mediating the effects that leptin has on reproductive endocrine blood markers. The association of leptin and reproduction to energy availability may indicate that they are simply independent correlates of energy availability. However, a more thorough discussion of the mechanisms linking leptin, reproduction, and fuel availability will explore this topic in greater detail.

Leptin might interact with a metabolic sensory system that regulated reproductive endocrine parameters in at least two ways. First, leptin synthesis and secretion in various tissues may be sensitive to metabolic fuel availability. Leptin may then act as a mediator of the metabolic signal and go on to regulate reproductive endocrine parameters, directly in the CNS/ovaries or indirectly. Second, leptin might affect reproductive endocrine parameters indirectly by regulating the metabolic signal that affects reproduction.

8. 1. Metabolic Signals Affect Leptin Levels, which can Affect Reproductive Endocrine Parameters

An energy deficit-induced decline in leptin may occur as a result of metabolic changes such as a change in fuel flux [75], an increase in free fatty acids (FFAs; [79, 80]) and ketones [78], a decrease in plasma glucose/insulin levels [81], and changes in glucose metabolism [82]. In turn, metabolically-induced changes in leptin may influence reproductive endocrine parameters.

Various metabolic factors may be involved in decreasing leptin levels with an energy deficit. A significant correlation between decreases in leptin occurring with a decrease in body weight and indicators of decreased glucose availability and increased lipolysis [71] suggest that change in fuel flux with an energy deficit may decrease leptin levels. The increase in free fatty acids (FFAs) may inhibit *ob* gene expression [79, 80], or have no immediate effect on leptin levels [133]. Although ketones, produced with fasting from FFAs, are suggested to decrease leptin levels [134], other research has shown no involvement [79].

Decreases in insulin may also influence the leptin response to an energy deficit. Insulin is a leptin secretagogue [81] and changes in leptin parallel changes in plasma insulin accompanied by weight loss [52]. Additionally, when serum glucose and insulin concentrations were clamped at basal levels, leptin did not change with a 72-hour fast [135]. The mechanism by which insulin exerts its effects on leptin mRNA expression and protein secretion may involve enhanced glucose uptake and metabolism [136]. Evidence of this is suggested by: the significant correlation of the decrease in leptin occurring with weight loss with a combined index of parameters representative of insulin action [75, 76]; decreases in adipocyte leptin mRNA and insulin-dependent leptin secretion when drugs that inhibit glycolysis or glucose transport are administered [137].

In contrast, other researchers have found that the adaptive response to an energy deficit and corresponding insulin actions do not regulate leptin levels [78, 138]. This is consistent with research finding that the decrease in serum insulin levels and leptin during an energy deficit were not significantly related [78, 138]. It may be that the effect of insulin or insulin effects on serum leptin concentrations depend on previous nutritional status [138] or the amount of weight loss [139]. It may be that the rate of glucose uptake into adipose tissue, rather than the prevailing insulin concentration, determines changes in circulating leptin as shown by in vivo studies in human subjects where different circulating insulin concentrations at constant glucose levels did not alter leptin levels [139].

Evidence that glucose metabolism affects leptin levels comes from research showing that a 57% increase in glucose availability, occurring as a result of the addition of exercise to a diet-induced energy deficit, results in a reduced suppressive effect of an energy deficit on the diurnal rhythm of leptin as compared to women who were restricted

using diet alone [96]. Inhibition of glucose oxidation significantly decreases plasma leptin concentrations in Syrian hamsters and adipocytes in culture [137].

The hexosamine pathway and its final product, uridine-5'-diphospho-N-acetylglucosamine (UDP-GlcNAc), may mediate the response of leptin to glucose [82] as shown by: an increase in leptin release in a culture of isolated subcutaneous adipocytes with glucosamine (an intermediate product in the UDP-GlcNAc bioynthesis); an increase in *ob* gene expression with infusion of UDP-N-acetylglucosamine, glucose, lipid, and uridine (all of which would be expected to increase the concentrations of the end-products of the hexosamine biosynthetic pathway; [140]); and a reduced glucose-stimulated leptin release when the rate limiting enzyme in hexosamine biosynthesis is inhibited [141]. Uridine-5'-diphospho-N-acetylglucosamine is utilized in the *O*-linked glycosylation of proteins including transcription factors. *O*-glycosylation has been shown to alter the transcriptional activity of Sp1, a transcription factor with binding sites in the *LEP* gene promotor [50], and increase leptin gene expression and serum leptin [142]. Thus, leptin synthesis and secretion may be influenced by the oxidative substrates that are part of the hexosamine biosynthetic pathway in tissues such as white adipose tissue and skeletal muscle.

Due to the complexity of neural and hormonal changes that occur with an energy deficit, the decreases in leptin that occur are probably due to a number of factors working together to regulate leptin. In addition to the metabolic factors mentioned, restricted intake increases sympathetic activity in adipose tissue with an energy deficit, which may decrease leptin production and increase fat mobilization through the beta-3-adrenergic receptors [143]. The extent of the involvement of these factors in regulating leptin during

an energy deficit may depend on differences in duration and severity of the energy withdrawal, the type of subjects studied, and initial leptin levels of the subjects. Thus, with an energy deficit, various metabolic factors decrease plasma leptin levels, which in turn, may modulate reproductive endocrine parameters, either directly, or indirectly.

8.2. Leptin Affects Metabolic Variables, which can Affect Reproductive Endocrine Parameters

Leptin may affect the metabolic response to energy deprivation, which in turn, may affect reproductive endocrine parameters. Ancillary hormones, such as insulin or cortisol, metabolic fuels (such as glucose or fatty acids), or enzymes (such as those involved in carbohydrate or fat metabolism) may function as signals of a shortage of oxidizable metabolic fuels, which may then be transmitted to the neural network at the central level of the reproductive axis (potentially through the vagus nerve; [129, 130]), thereby resulting in suppression of reproductive function [34]. Thus, leptin may work in concert with other hormones or metabolites to signal to the brain about the depletion of energy stores so that the appropriate physiological response can be initiated. Demonstration of the impact that leptin has on metabolic variables can be shown through effect of leptin on metabolic rate, fuel oxidation, and enzymatic changes, especially with an energy deficit.

8.2.a. Leptin and Metabolic Rate

Evidence that leptin affects the metabolic response comes from studies evaluating the effect of leptin on metabolic rate and fuel oxidation. Some researchers have found

that changes in leptin were significantly correlated to changes in resting energy expenditure with weight loss [41], while other researchers have found no relationship [146-147]. Leptin may increase metabolic rate under energy replete conditions by: stimulating substrate cycling as shown by an increased ratio of glycerol:FFAs released from cells with leptin treatment [148]; increasing the proton leakiness of membranes through the affect of leptin on triiodothyronine [149]; increasing the proton leakiness of membranes [150-151] by increasing the sympathetic nervous system-mediated mRNA expression and membrane concentration of uncoupling protein-1 (UCP-1; the only UCP that has been clearly shown to increase the thermogenic capacity of animals [152-153]. When energy intake is inadequate, lower leptin levels would potentially result in a decreased metabolic rate, less substrate cycling, and less mitochondrial oxidative phosphorylation. These metabolic changes may be signals to the reproductive axis that oxidative capacity is decreased, which in turn may result in a suppression of reproductive endocrine parameters.

8.2.b. Leptin and Oxidizable Fuels

Leptin-induced changes in contribution of different oxidative fuels with an energy deficit may be signals to the reproductive axis that oxidative capacity is decreased, which in turn may suppress reproductive endocrine parameters. This is suggested by the finding that anovulation in lean Syrian fasted hamsters, is not reversed through exogenous leptin administration when methyl palmoxirate (MP: inhibits fatty acid oxidation) and 2-deoxy-s-glucose (2DG: inhibits glucose utilization) are administered [102]. Thus, the reversal of fasting-induced infertility by exogenous leptin treatment may be due to indirect effects

of leptin on fuel oxidation or utilization [102]. Leptin may not be able to facilitate reproductive function beyond the animal's ability to hydrolyze, oxidize, and utilize these fuels [102]. This may explain why: leptin can fully reverse the effects of fasting-delayed puberty in rats restricted to 80% of their *ad libitum* food intake, but not in rats restricted to 70% of their *ad libitum* intake [154]; leptin increases the length of lordosis in *ad libitum*-fed, but not in energy restricted hamsters [14]; leptin decreases the duration of lactional diestrus in rats who were acutely energy restricted, but not in rats who were chronically energy restricted [87]; leptin administration attenuates, but is not able to completely overcome the effects of fasting on the estradiol-induced LH and prolactin surges in rats [155]; and why exercise (which is similar to leptin treatment in that it promotes fuel oxidation and decreases energy storage) actually counterbalances the effects of various environmental inhibitors of puberty, gonadal regression, gonadotropin secretion, anestrus, and estradiol-enhanced sex behavior [156-158]. These results suggest that exercise and leptin treatment facilitates reproductive function, probably by increasing oxidation and intracellular availability of metabolic fuels.

8.2.c. Leptin and Lipid Metabolism

Leptin may signal the reproductive axis of decreased energy reserves available for reproductive function through its effects on lipid metabolism. Leptin may inhibit fatty acid synthesis through a reduction in activity and mRNA for enzymes in FA synthesis, and promote fatty acid lipolysis and oxidation.

Leptin treatment results in increased rates of FFA oxidation and lipolysis, while reducing the rates of esterification within a number of tissues such as pancreatic islets

[13], skeletal muscle, and adipose tissue. Leptin treatment increases mRNA for enzymes of FFA oxidation [148, 153], upregulates and increases activity of carnitine palmitoyl transferase-1 (CPT-1 [159]), and decreases mRNA for an enzyme known to inhibit CPT-I activity [153]. In mice, systemic leptin treatment results in increased mRNA expression of thermogenic proteins [149], lipoprotein lipase [169], and hormone-sensitive lipase [160]. Leptin stimulates intracellular lipolysis within the adipocyte as shown by a leptin-induced disappearance of body fat without a rise in FFAs [148]. Free fatty acids that are released from lipolysis within the adipocytes remain in the adipose tissue and are oxidized there, versus going to the liver and being oxidized [148].

Although the interaction between leptin and insulin is controversial [161], leptin may mediate lipid metabolism indirectly by reducing the lipogenic effects of insulin in adipocytes and skeletal muscle [149]. Leptin administration results in reduced insulin levels, which in turn mediates: decreases in LPL activity, plasma TG concentration, and liver TG content in lean and *ob/ob* mice; and decreases in lipid oxidation and synthesis in mouse skeletal muscle [162]. It appears as if high leptin levels *in vivo* depress the lipogenic effect of insulin [149].

The effect of leptin on lipid metabolism may also be mediated by interactions that leptin has with other hormones. Leptin stimulates glucagon release, which is a stimulator of lipolysis [163]. Leptin receptors have been found in the adrenal medulla and leptin has been shown to increase lipolysis by stimulating the synthesis and release of epinephrine and norepinephrine from cultured chromaffin cells [164]. Leptin may also stimulate lipolysis through an action of leptin on the ventromedial hypothalamus to activate the sympathetic outflow, which may increase lipolysis through the adrenergic pathway [165,

166]. The stimulatory effect of leptin on GH release contributes to the lipolytic effect of leptin as GH also has lipolytic activity [149].

Thus, leptin may inhibit TG synthesis and stimulate fatty acid lipolysis and oxidation, potentially by suppressing insulin, thereby suppressing the lipogenic effects of insulin. The exact mechanism by which leptin has these effects on lipid metabolism, may also involve other intermediates. When leptin levels are decreased with an energy deficit, FA lipolysis and oxidation may decrease, while FA synthesis may increase. These changes in lipid metabolism may in turn, serve as cues to the reproductive axis about the amount of energy available to maintain reproductive hormone profiles and function.

8.2.d. Leptin and Carbohydrate Metabolism

Leptin may signal the reproductive axis of decreased energy reserves available for reproductive function through its effects on carbohydrate metabolism. Although leptin was shown to exert a direct and acute insulin-like effect on glucose metabolism [167], its effects on glucose metabolism are extremely variable. In adipocytes, leptin has been shown to have no effect [168] or an inhibitory [169] effect on insulin-stimulated glucose metabolism [170-171]. Similarly, in skeletal muscle, leptin has been reported to stimulate [172], inhibit [173], or have no effect on [174-175] glycogen synthesis; to have or have no effect on glucose oxidation [175]; and to stimulate [172, 176], inhibit [168], or have no effect on [174, 177] glucose transport. Leptin has been found to decrease glucose oxidation [178], increase glucose oxidation [167], or have no effects on glucose oxidation at rest in rat soleus muscle [179]. When food-deprivation-induced anestrus is prevented by leptin treatment, leptin-treated hamsters show a profile of circulating

metabolic fuels that suggests that leptin treatment increases the uptake of peripheral glucose [13].

Leptin may affect glucose metabolism through its effects on insulin. Although leptin inhibits insulin [180], leptin increases insulin sensitivity in normal rats both under fasting conditions and in the presence of hyperinsulinaemia under conditions of clamped glycaemia [81]. As a result, leptin may promote some insulin actions [172] possibly in concert with insulin, and may inhibit [175] or leave some insulin actions unaffected [162]. The involvement of insulin in the regulation of glucose metabolism by leptin may depend on the tissue type and insulin action examined.

Overall, effects of leptin on glucose metabolism or insulin-mediated glucose metabolism are extremely controversial. Differences in results may be due to differences in: experimental approach used; age, species and strains of animals used; type of culture used (ie culture versus native tissue); type of diet; size and duration of the leptin dose; and the degree of leptin or insulin resistance in the animal model being studied. Further studies are needed to clarify the relationship between leptin, insulin, and glucose metabolism under energy replete and deplete conditions.

Based on the stimulatory effects of leptin on glucose metabolism under energy replete conditions, decreases in leptin levels occurring with an energy deficit may send signals to the reproductive axis about the amount of energy available for reproductive function via leptin-induced changes in carbohydrate metabolism. The effects of low leptin levels on aspects of carbohydrate metabolism are much more difficult to predict because the effects of leptin on glucose metabolism are so controversial. Generally, it appears as if leptin or insulin may affect reproductive function by modulating the

availability of glucose at the hypothalamic or extra-hypothalamic sites, instead of or in addition to their direct actions on neurons that control GnRH secretion [13]. Regardless of how leptin affects carbohydrate metabolism, metabolic signals of carbohydrate metabolism may reach the reproductive axis through other hormones, through change in metabolite levels, or through sympathetic or parasympathetic afferents, alerting it to the availability and opportunity for oxidation of body fuels. Future research is required to determine how leptin translates metabolic changes occurring as a result of changes in carbohydrate metabolism with an energy deficit, to the reproductive axis.

8.3. The HPA Axis, Leptin, and Reproductive Endocrine Parameters

Leptin may affect reproductive endocrine parameters by acting on components of the HPA axis. With an energy restriction, leptin may decrease the effectiveness of the actions of cortisol. This would reduce the suppression that the HPA axis exerts on the release of GnRH and gonadotropins. It is unlikely that changes in the HPA axis with an energy deficit may affect reproductive function by affecting leptin. The glucocorticoid-induced stimulation of leptin expression [180] is not consistent with increases in cortisol [166] and decreases in leptin seen with an energy deficit [52, 70, 71].

Leptin may suppress the HPA axis. In rodents, exogenous leptin administration clearly suppresses starvation-induced HPA activation [97]. This is consistent with the finding that inverse relations between 24-hour fluctuations in plasma levels of leptin and cortisol, as well as between leptin and ACTH, were found [180]. Previous research has shown that leptin treatment of mice substantially blunts the stress-induced rise in ACTH and corticosterone and that leptin inhibits the hypoglycemia-induced CRH secretion by

perifused rat hypothalamus [181]. The physical relationship between the location of the long form of the leptin receptor in the arcuate nucleus and the CRH-producing neurons in the paraventricular nucleus also suggests that leptin may regulate the HPA axis [166]. However, other studies have found no inhibitory effect of leptin on cortisol release [107, 182].

Leptin may affect the HPA axis by affecting glucose availability [182]. Leptin is known to enhance energy utilization in tissues [183, 184]. Thus, the decline in glucose availability with an energy deficit could serve to activate the HPA axis, as evidenced by the increase in circulating ACTH and corticosterone [185] and depression in LH secretion [186] during transient glucoprivation produced with a competitive antagonist of glucose [187]. However, the possibility of a direct interplay between leptin and the HPA axis cannot be excluded.

Leptin may counteract the increase in the HPA axis activity during fasting to restore gonadotropin secretion. This is shown when exogenous leptin, administered to ob/ob mice, as well as fasting wild-type mice, ameliorates hypercortisolemia [105, 176] and infertility [3, 61, 100, 105]. In contrast, leptin infusion did not affect reproductive function through the HPA axis in male rhesus monkeys who had been fasted 42-57 hours [116]. Contradicting results on the effect of leptin on the HPA axis may be due to the type of stress imposed. It is suggested that the effect of leptin on the HPA axis is dependent on the type of stress, where exogenous leptin can suppress the HPA axis in response to a metabolic stress (glucoprivation), but not in response to a non-metabolic stress (immobilization) [182]. Further research is required to clarify whether leptin

suppresses the HPA axis, and whether this suppression results in an enhancement in reproductive function.

8.4. Summary of Indirect Effects

Although leptin has been shown to directly modulate GnRH [50] and LH/FSH [104] synthesis and secretion with an energy deficit, it appears more likely that leptin regulates reproductive endocrine parameters at the peripheral level through interaction of leptin with metabolites or by affecting activity of the HPA axis. Leptin might interact with a metabolic sensory system that controls reproduction in at least two ways. First, leptin synthesis and secretion in various tissues may be sensitive to increases in free fatty acids and ketones, decreases in plasma glucose/insulin levels, and changes in glucose metabolism (ie hexosamine pathway) occurring with an energy deficit. Leptin may then act as a mediator of the metabolic signal and go on to regulate reproductive endocrine parameters, directly in the CNS/ovaries or indirectly. Second, leptin might affect reproductive endocrine parameters indirectly by regulating the metabolic signals such as insulin, glucose, fatty acids, enzymes in carbohydrate and lipid metabolism, or activity of the HPA axis, that affect reproduction.

9. Conclusion

Reproductive disorders associated with low energy intake and high energy expenditure are frequently observed in female athletes, women with an eating disorder, and women engaging in disordered eating behaviors. The etiology of these reproductive disorders remains unknown, although recent evidence suggests that the effect of diet and

exercise on reproductive function is mediated by its impact on metabolic fuel availability. There is little evidence to support the hypothesis that a critical amount of body fat is required for normal reproductive function, however adipose tissue stores may affect reproductive function through its involvement in energy balance [188]. The identity of an afferent system that senses energy availability, which in turn, provides this information to the neural network of the reproductive tract, has not been identified. Yet, the recent discovery of the adipocyte-derived peptide hormone, leptin, may serve as a potential peripheral signal relating nutritional status and reproductive function. This is consistent with the findings that leptin decreases with an energy deficit, and that leptin affects reproductive function.

Leptin may decrease with an energy deficit as a result of metabolic changes that occur with an energy deficit. Leptin may affect reproductive endocrine parameters through the metabolic sensory system by acting as a mediator where leptin may respond to a metabolic signal and affect reproductive endocrine parameters itself (directly or indirectly), or may affect metabolic signals (such as the HPA axis hormones), which may then affect reproductive function.

Although animal studies demonstrate causal links between leptin and reproductive endocrine parameters, human studies show, at best, correlational relationships between these variables. Thus, the causal link between leptin and reproductive endocrine parameters remains unestablished in humans. Even if leptin does have a role in regulating reproductive endocrine parameters by acting as a metabolic signal, it is not the only signal that mediates reproductive function through the metabolic sensory system.

This suggests that leptin may be one of many variables that respond to changes in energy availability and affect reproductive endocrine parameters.

Various factors affect weight loss success. In turn, weight loss, may affect reproductive endocrine blood parameters. The hypotheses tested by this research were:

1. A decreased energy availability in eumenorrheic obese women results in suppressed reproductive endocrine blood markers and decreased leptin levels.
2. Leptin mediates changes in the reproductive axis when energy availability is decreased, through cortisol.

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Chapter I

Literature Review: Part B

1. Introduction

From the previous literature reviewed, weight loss in obese eumenorrheic women, who are not insulin resistant or diabetic, doesn't compromise reproductive function, and may actually improve reproductive function (1, 2). Additional benefits to weight loss in obese persons are numerous, due to the health risks associated with obesity, including hypertension (3), hypercholesterolemia, non-insulin dependent diabetes (4), coronary heart disease, congestive heart failure (5, 6), certain cancers (7), sleep apnea and respiratory disorders (8), gallstones (9), depression (10), and arthritis (11). Weight reduction as little as 10-15% of body weight has been shown to provide some reduction in health risk (12).

Thus, obesity, "an excess of body fat frequently resulting in a significant impairment of health" (13, p. 157), is a major public health problem. Obesity is a result of a positive energy balance, where energy intake is greater than energy expenditure (14). Body mass index is used to classify obesity where obesity is defined as having a body mass index (BMI, that is the ratio of weight (kg) to height (m) squared) greater than 30 kg/m^2 , and overweight is defined as having a BMI greater than 25 kg/m^2 (15).

The prevalence of overweight and obesity (body mass index (BMI) > 25 kg/m^2) among Canadian adults in 2001 was approximately 50% (16), while the prevalence of overweight and obesity (body mass index (BMI) > 25 kg/m^2) in those aged 20-64 years in the United States between 1988-1994 was approximately 60% and 50% for men and women respectively (17). When obesity (defined as having a BMI > 25 kg/m^2)

prevalence is broken down according to age, researchers found that 43% and 33% of men and women aged 20-29 years were obese, and 58% and 47% of men and women aged 30-39 were obese (15). Thus, obesity appears to be a major health risk for a large number of North Americans.

2. Success of weight loss programs

Dieting is one of several approaches to combat obesity, yet despite the billions of dollars spent on weight reduction programs, their success is questionable (17). Weight loss success has been defined through the evaluation of weight changes in weight loss programs as well as through the evaluation of attrition rates in weight loss programs. Only one third of 4200 and 2300 weight loss participants, assessed using two major surveys, were found to be successful dieters (defined as having lost enough weight to move from a BMI value greater than 25 kg/m² to a BMI of 20-25 kg/m²; 18). Attrition rates for weight loss programs are very variable and range from 23-64% for clinic-based weight loss programs (19-22), and 50-95% for commercially-based programs (19). However, a more recent analysis of attrition rates for weight loss programs in the last 10-15 years is determined to be approximately 20% (23). Variable attrition rates may be due to differences in weight loss programs evaluated, the subjects studied, and the environmental context surrounding weight loss (ie if there was financial reimbursement; if there was a large health risk etc).

3. Characteristics of successful and unsuccessful dieters

Given the large number of people trying to lose weight, the characteristics of successful and unsuccessful short- and long-term outcomes remains an important goal to optimize program results. A discussion of characteristics of successful and unsuccessful dieters also reveals barriers associated with weight loss and strategies utilized for weight loss.

3.1 Self-concept

Successful dieters were found to have a positive self-concept (24). This is in contrast to unsuccessful dieters who were found to possess a negative self-concept characterized by unrealistic expectations for weight loss, greater body dissatisfaction, low self-efficacy, and a higher number of recent dieting attempts (24). A higher perceived stress from one's weight may manifest itself through the creation of unrealistic goals (24). Unrealistic expectations related to the ease, speed, likely degree of change, and presumed advantages associated with making changes may overpower knowledge of one's prior failures (25).

Past weight loss experiences may affect the success of weight loss on an emotional level because participants' present attributions may reflect past experience (26). However, the effect of previous weight loss attempts on weight loss success is controversial as previous research has found that previous weight loss attempts can either impede (27, 28) or facilitate weight loss (29). The discrepancy between these results may reflect the methods of sampling used or may indicate that weight loss is the result of many years of perseverance.

Body dissatisfaction is associated with increased drop out rates (30) in weight loss programs. Body dissatisfaction for the obese person may be perpetuated by the biases and cultural prejudices expressed in the health care system and exercise environment (20) towards overweight/obese people that characterize obese people as lazy, stupid, dishonest, and lack ambition (31). Years of social stigmatization can affect an obese person's acceptance to the label (32), affect the behaviors associated with the label (32), decrease body dissatisfaction (33), and increase social physique anxiety (33). Obese/overweight women may seek less threatening strategies for weight loss that do not involve exercise, or do not involve exercising in a public setting (33).

The distaste and distortion of their own body size may prevent (33) or encourage (34) obese individuals to seek weight loss. Motivation to lose weight for reasons of improving body satisfaction or body size can lead to unhealthy weight loss strategies, such as binge eating (35), or excessive exercise (36), because too much emphasis is placed on beauty versus health (37). Body dissatisfaction as a motivation for weight loss may lead to short-term fixes, which cause reversion back to old habits, and yo yo dieting (37).

3.2. Stress and problem-solving ability

Experiencing stressful life events can make weight loss efforts more difficult. A greater percentage of weight loss participants who had lost weight, but regained it, reported the occurrence of major illnesses, bereavements, and other environmental stressors at the end of treatments as compared to weight loss participants who were able to maintain their weight losses (38). Other researchers suggest that it is not stress that

determines success of the weight loss participant, but rather the ability to cope with stress (39). Various studies show that weight loss participants (40, 41) and weight loss maintainers (42, 43) are able to generate coping responses to more situations and use effective problem solving to cope with stress.

Rather than managing problems directly, unsuccessful weight loss participants report using "escape-avoidance" ways of coping such as eating, sleeping more, or wishing the problem would go away (44). This is in contrast to successful dieters who are more likely to attribute problem solving to strategies (such as meal plan) that were more personally controllable, and to confronting behaviors, like calorie balancing skills (45). Thus, enhanced problem solving skills may characterize a successful weight loss participant.

3.3. Support

In a weight loss program, adequate support can facilitate the management of difficult situations, such as gender-role conflicts (which involve childcare and household duties), and lack of time. This was suggested by the observation that spousal, social, and familial support (46-50) improve diet and exercise adherence and enhance weight loss. The basic premise behind support is that family and friends, if cognizant of appropriate eating/exercise habits and skills, may model, suggest, support, reinforce positive behaviors for the individual trying to lose weight (47), help weight loss participants learn greater self-acceptance, develop new norms for interpersonal relationships, and help manage stressful work or family-related situations (51).

Spousal and family support with childcare and household responsibilities, especially for women in the work force, can facilitate regular exercise and weight loss (52). Gender role conformity, which is a socialization pattern that emphasizes other-directedness where a woman has a sense of responsibility to other than to self (53), acts as a powerful barrier to leisure and physical activity for adult women. Women often do not feel as if they deserve to engage in leisure or physical activity, and do not have the time or the energy to experience it because of their many obligations such as homemaker, mother, and/or breadwinner (53). Support may override the guilt associated with committing time to one's own needs, versus the needs of the family. Thus, support not only facilitates weight loss management by overcoming gender role conformity, but also provides time to engage in weight management behaviors, as time has been cited as the main barrier to weight loss (54, 55).

Social support during exercise may also facilitate the development of a regular exercise program (52, 56). The idea that increased support for or during exercise increases exercise regularity is not surprising given the visible and interactive nature of the exercise context, the reorganization of lifestyles, sometimes considerable courage and, a good deal of mental and physical effort (33). Overall, support structures offer great potential for the overweight and obese women who face considerable barriers to weight loss behaviors.

3.4. Exercise inclusion in weight loss

Successful dieters are also characterized by being regularly active (57). Although a number of studies evaluating the effects of weight loss with diet versus diet plus

exercise have found that weight loss attributable to increased physical activity is small (58, 59), physical activity has been shown to be one of the key correlates of sustained weight loss (60-62). This may be due to improvements in psychological functioning that result from exercise (for example, improved body image, psychological well-being, and eating self-efficacy), which may help obese people better cope with stressful situations and reduce overeating and the resultant negative emotions that lead to relapses (60, 63).

Time barriers for physical activity were the most frequently reported by both men and women across adult years (64). Weight loss participants successful at weight loss incorporated regular physical activity into their routine by setting realistic goals, making exercise a priority, exercising consistently but not for long extended periods of time that would impact other aspects of life, and acknowledging that changes occurring as a result of exercise would take time (37).

Decreases in the daily living activities due to technological advances, may make it more difficult for obese persons to lose weight. Technological advances like the phone, the remote control, the elevator, the escalator, the car, and the computer result in less physical activity (37). Although the contribution of daily living activities to weight loss is not known, research shows that increases in daily living activities can increase exercise adherence in those attempting to lose weight (51).

3.5. Eating practices

Successful dieters make time for eating changes, and as a result, are able to make healthier nutrition choices using a variety of methods (37). In contrast, unsuccessful dieters spend less time making healthy nutrition decisions, ingesting higher calorie food

items, snacking more frequently, and eating out more (65). Successful dieters learn to adapt their eating pattern to their lifestyle in order to lose weight (65) by putting more time and effort into changing eating behaviors (66) by creating strategies for handling cravings for high fat and sweet foods (67), planning meals for the day (37), eating out less to avoid eating unhealthily on the run (37, 65), recording intake (66), monitoring weight (66), attending educational sessions promoting weight loss (49, 66, 68), and reading food labels (37). Thus, successful dieters are able to make healthy nutrition choices, even with a hurried and stressful lifestyle (69).

3.6. Self-efficacy

Self-efficacy, or the belief that one is capable of performing the necessary behavior required to bring about a desired outcome (69), is positively associated with weight loss (69-71), and decreased attrition rates in weight loss programs (71). A weight loss participant will more likely take the necessary action to attain a goal if they have high self-efficacy (70) or develop high self-efficacy as a result of success associated with weight loss (72). The greater the perceived difficulty of adopting a particular activity, the lower the likelihood of adoption (73). Regular exercise may have a positive impact on self-efficacy due to changes in perceived fitness and satisfaction with physical shape and weight (74). Increases in self-efficacy with weight loss may facilitate the identification and management of difficult situations (75). Thus, a high self-efficacy, or the development of a high self-efficacy, can facilitate weight loss by improving the ability to overcome difficult and challenging situations.

4. Social Cognitive Theory

Based on the multiple, interacting factors shown to be associated with weight loss success, Social Cognitive Theory (SCT) may provide a model from which to investigate weight loss success. Social Cognitive Theory explains behavior in terms of a triadic, dynamic, and reciprocal model in which behavior, personal factors, and environmental influences all interact (74). Health educators and behavioral scientists have used SCT concepts to develop procedures or techniques that affect these underlying cognitive variables, thereby increasing the likelihood of behavioral change. In this way, SCT not only explains how people achieve and maintain certain behavioral patterns, but also provides the basis for intervention strategies.

Social cognitive theory also incorporates how self-efficacy (SE) affects behavior changes, which has been suggested to have a role in determining weight loss success (71). Self-efficacy was suggested to be the most important prerequisite for behavioral change, because it affects how much effort is invested in a given task and what level of performance is attained. Repetition of the performance of a single task builds a person's SE by changing the person's performance expectations. Through repeated successful enactment of progressive tasks, the person acquires enhanced expectations of success in the task, which in turn, affects task persistence, initiation, and endurance, and thus promotes behavioral changes (76).

Self-efficacy also has an important role in self-control, which in turn affects a person's selection of the extent of behavioral change and his/her practice in building confidence in self-regulation (76). Self-control has a large impact on whether a goal is achieved (76). Thus, if SE is high, self-control will also be high, and the person will

most likely achieve their goals. However, if SE is low, self-control will also be low, and the individual has a lesser probability of achieving his/her goal.

5. Conclusion

Obesity prevalence appears to be increasing. Although, dieting is one approach implemented to combat obesity, its success is questionable. Given the large number of people trying to lose weight, the characteristics of successful and unsuccessful short- and long-term outcomes remains an important goal to optimize program results. Although the studies evaluating characteristics of successful and unsuccessful dieters are limited by the variable definitions of what a successful dieter is (77), distinct differences between successful and unsuccessful dieters have been documented. Successful dieters appear to have a positive self-concept, support for change, exercise consistently, manage time, have high levels of self-efficacy, and have good problem solving skills. Social cognitive theory may provide a suitable model to investigate weight loss success because this theory incorporates behavioral, personal, and environmental factors discussed above and involved in weight loss. Additionally, SCT incorporates how SE affects behavior change.

Due to the numerous factors affecting weight control efforts previously described, this study sought:

A. To determine if there were three types of participants in the weight loss program described in hypotheses #1 and #2 (those who lose weight, those who do not complete the weight loss program, and those who complete the program, but are unable

to lose weight) through the analysis of diet counseling data already collected from the weight loss program described in hypotheses #1 and #2.

B. To explore and describe challenges, strategies and facilitating factors influencing eating and exercise patterns of participants able to lose weight, participants who do not complete the weight loss program, and participants who complete the weight loss program, but are unable to lose weight, through the analysis of diet counseling data already collected from the weight loss program described in hypotheses #1 and #2.

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Chapter II Research Plan

Rationale

Extensive weight loss can result in reproductive irregularities in women, such as hypothalamic amenorrhea. Aside from the obvious negative impact on reproductive success (ie infertility), amenorrhea places women at increased risk for reduced bone density and fracture risk, increased breast cancer risk, and increased cardiovascular disease risk (1).

A decreased energy availability (EA; the net energy available to the body after accounting for energy intake and energy expended) has been suggested to be a cause of amenorrhea, where an energy deficit created by diet and/or exercise may compromise menstrual function in order to fulfill "essential energetic priorities". This 'energy availability hypothesis' is supported by the recent discovery of an adipocyte derived peptide hormone, leptin. Leptin may provide a metabolic/neuroendocrine linkage between EA and reproductive function by affecting cortisol. Thus, information obtained about weight loss, leptin, and reproductive function will have clinical relevance for women with menstrual irregularities, which includes athletes, women with chronic stress, and women with eating disorders. Manipulation of leptin levels through diet and exercise, may have a role in normalizing menstrual function, which in turn, may minimize the adverse health effects associated with amenorrhea in women. Additionally, leptin could potentially be used as a pharmacological agent to treat menstrual disorders.

A loss of 10% of body weight was chosen for the present study as this has previously been suggested to result in a significant decrease in plasma leptin (2), which

may then have effects on reproductive function. Obese women were used to study the effects of weight loss on reproductive function because loss of 10% of body weight does not significantly compromise reproductive function in this population (3), but does result in changes in luteinizing hormone (LH; 4). This is consistent with results showing that a 72-hour fast in normal weight women resulted in transient alterations in luteinizing hormone without an impairment in ovulatory and reproductive function while a 72-h fast in lean women resulted in profound decreases in LH, anovulation, and a lengthened follicular phase (4).

Weight loss in obese eumenorrheic women, who are not insulin resistant or diabetic, doesn't compromise reproductive function, and may actually improve reproductive function (3, 4). Additional benefits to weight loss in obese persons are numerous, due to the association of obesity with physical problems such as heart disease, cancer and diabetes (5-7), as well as its more controversial link with psychological problems such as depression and low self-esteem (8). This has led to the development of weight reduction programs, as the loss of as little as 10-15% of body weight has been shown to provide some reduction in health risk (9).

The differences between people who do and do not lose weight in these programs may be due to specific characteristics, skills, and level of readiness. Recently, two groups of dieters have been identified which include participants who do not complete the program and participants who may complete the program, but are unable to lose weight (10). Previous characterization of these two groups of dieters unable to lose weight has not been documented. Comparison of the characteristics, barriers, skills and contextual factors surrounding weight loss by those who lose weight, those who aren't

able to complete the program, and those who are able to complete the program but don't lose weight may allow for a better matching of interventions to participants, saving resources, and increasing program efficacy.

Qualitative methods were used to elicit weight loss participants' experiences articulated in their own words within the context of their personal environment. Documentation of participants' actual experiences during counseling sessions provided immediate feedback on weight loss interventions and participants' personal experiences including unanticipated influences on success. These factors have all been previously cited as valid reasons for use of qualitative research designs (11, 12).

Based on this rationale, the following is hypothesized for Study #1:

Hypothesis #1

A decreased energy availability in eumenorrheic obese women results in suppressed reproductive endocrine blood markers and decreased leptin levels.

This hypothesis was tested by achieving the following objectives:

A. The effect of an energy restriction, involving both diet and exercise, in eumenorrheic obese women on serum luteinizing hormone (LH) concentrations, leptin concentrations, and leptin pulsatility features was examined before and after loss of 10% of body weight.

B. The effect of an energy restriction on the sensitivity of the hypothalamic-pituitary-ovarian axis was assessed through the response of the anterior pituitary (the release of LH) to exogenous gonadotropin releasing hormone (GnRH) before and after 10% weight loss in obese eumenorrheic women.

C. To determine if changes in leptin are related to hormonal changes occurring with an energy restriction. Specifically, the following relationships were evaluated: leptin and LH concentrations, leptin area under the curve, and pulsatility parameters and response of LH to the GnRH bolus.

Hypothesis #2

Leptin is related to changes in reproductive endocrine blood markers when energy availability is decreased, through cortisol.

This hypothesis was tested by achieving the following objectives:

A. To determine if changes in plasma leptin were related to changes in 24-hour urinary cortisol following 10% loss of body weight in obese eumenorrheic women.

B. To determine if changes in plasma LH were related to changes in 24-hour urinary cortisol and/or urinary cortisol and plasma leptin (as shown by multiple regression) following of 10% loss of body weight in obese eumenorrheic women.

Research Questions for Qualitative Study (Study #2)

A. To determine if there were three types of participants in the weight loss program described in hypotheses #1 and #2 (those who lose weight, those who do not complete the weight loss program, and those who complete the program, but are unable to lose weight) through the analysis of diet counseling data already collected from the weight loss program described in hypotheses #1 and #2.

B. To explore and describe challenges, strategies and facilitating factors influencing eating and exercise patterns of participants able to lose weight, participants who do not complete the weight loss program, and participants who complete the weight loss program, but are unable to lose weight, through the analysis of diet counseling data already collected from the weight loss program described in hypotheses #1 and #2.

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Chapter III

Weight loss in moderately obese eumenorrheic women decreases leptin levels, leptin pulsatility, LH levels, and hypothalamic-pituitary responsiveness of the reproductive axis.

Introduction

The hypothalamic-pituitary-ovarian (HPO) axis governs reproductive function and is sensitive to changes in physiological environment. Factors such as exercise and nutrition, can alter reproductive function. Sensitivity of the reproductive axis to change in nutrition and energy expenditure is demonstrated by the high incidence of reproductive disorders observed in women attempting to lose weight (ie anorexia nervosa; 1), and in women who are very athletic (2). The etiology of nutrition- and exercise-related reproductive disorders remains unknown. A number of causative factors have been suggested including negative energy balance, the stress of exercise weight loss, and extreme leanness. Although the critical-fat hypothesis is not supported (3), it continues to receive attention. Body fat is indirectly involved in regulating reproduction through its role in determining energy availability (the net energy available to the body after accounting for energy intake and energy expended; (2), where reproductive function is dependent on energy availability (4).

Identification of the adipocyte-derived *ob* gene product, leptin, restored interest in the relationship between fat and fertility. The lipostatic hypothesis suggests that leptin affects reproductive function through a link with adiposity (5). This is supported by the observation that very obese *ob/ob* mice are infertile due to deficiency of leptin (6). Parallel changes in the levels of body fat, plasma leptin concentrations, and plasma LH

concentrations with weight loss indicate that leptin is related to reproductive function through body fat stores during energy imbalance.

However, more recent findings suggest that low energy availability rather than inadequate body fatness is likely the mechanism which impairs hypothalamic-pituitary-ovarian function in women (4). The peripheral signal that informs the neural network of energy available for reproductive function has been suggested to involve leptin. Thus, leptin mediates: correction of fertility in male and female *ob/ob* mice (6); acceleration of puberty in normal female mice (7), rats (8), and food restricted rats (9); and partial restoration of LH levels in malnourished rats (10), through a mechanism involving oxidizable metabolic fuels. The metabolic hypothesis, in which leptin links metabolic energy availability to reproduction, represents a much more realistic mechanism to explain the role leptin has in regulating reproductive function due the problems associated with the link between body fatness and reproductive function (3).

Since the integral function of the HPO axis under conditions of energy equilibrium appears to be influenced by leptin, it is suggested that reproductive dysfunction during periods of energy imbalance may also be linked to leptin. With an energy restriction, leptin may function as a 'barometer' of the body's physiological state that sends signals to the hypothalamic-pituitary axis of the reproductive tract. Evidence of this comes from studies finding that leptin levels are low in subjects with reproductive hypothalamic abnormalities such as amenorrhea (1) and anovulation (11). Leptin may affect the GnRH pulse generator as demonstrated by evidence showing that leptin stimulates GnRH release from hypothalamic explants (12), increases LH/FSH levels in male *ob/ob* mice (13), increases basal LH level in female *ob/ob* mice (6), and improves

LH pulsatility characteristics in monkeys fasted for 48 hours (14). The mechanism by which leptin affects the GnRH pulse generator response to an energy restriction may be through the effect of leptin on sensors of energy availability or the hypothalamic-pituitary-adrenal (HPA) axis, which may then affect reproductive endocrine parameters. Alternatively, lipostatic sensors or sensors of energy availability may influence leptin, which may then affect reproductive endocrine parameters directly or indirectly.

Given the well-documented effects of metabolic fuel availability on reproduction (2, 4) and the effect of leptin on fuel oxidation (15), it is possible that leptin affects reproduction through an interaction with metabolic signals and/or detectors. This is suggested from results finding that the influence of leptin on reproduction is eliminated by chemicals which inhibited fuel oxidation in lean Syrian hamsters (15). It is also possible that changes in energy availability, such as decreases in carbohydrate availability (2) change leptin levels or tissue sensitivity to leptin. Leptin may then affect reproductive endocrine parameters directly (13) or indirectly through intermediate neuropeptides (16). If leptin is involved in mediating the effects of metabolic energy availability on reproduction, then changes in plasma leptin would be more acutely responsive to changes in availability of metabolic fuels than to overall levels of body adiposity. Specifically, the metabolic hypothesis would predict that with weight loss, a link would be seen between: energy availability and plasma LH; plasma LH and plasma leptin; and plasma leptin and energy availability.

The HPA axis and leptin response to weight loss may also further our understanding of the role that leptin has in regulating reproductive endocrine parameters. Increase in ACTH and corticosterone concentrations in leptin-deficient *ob/ob* mice (17),

suppression of the starvation-induced HPA activation (10) in rodents with exogenous leptin administration, and the blunted stress-induced rise in ACTH and corticosterone in rodents administered leptin (10, 17), raise the possibility that leptin and the HPA axis may interact to alter reproductive endocrine parameters. Changes between leptin and the HPA axis are associative at best, but it is feasible that leptin may dampen the increased cortisol levels often observed with an energy restriction, thus, lessening suppression exerted by the HPA axis on release of GnRH and gonadotropins. Leptin treatment of the *ob/ob* mouse, as well as the fasting wild-type mouse, ameliorates both hypercortisolemia (10, 17) and infertility (6, 10). If leptin affects reproductive endocrine parameters, in the current study, through the HPA axis with an energy restriction, then urinary cortisol levels would be elevated, which would suppress plasma LH (as evident by an inverse correlation between urinary cortisol and serum LH). Additionally, plasma leptin would be inversely related to urinary cortisol, and plasma leptin would be directly related to serum LH.

The purpose of this study was to examine the effects of weight loss in obese eumenorrheic women on serum leptin, anterior pituitary sensitivity to a GnRH bolus, and urinary cortisol.

Experimental Subjects

Healthy, eumenorrheic (defined as having menstrual cycles that occur at intervals of 25-38 days (determined through evaluation of cycle length 2-3 times throughout the study duration; (18)), non-smoking, obese, women were recruited for a long-term weight loss program. The subject criteria included: being sedentary for the past year (they did not exercise more than 20 min each week; assessed using a history of previous activities

(19)); having a BMI between 30-40 kg/m², which was indicative of moderate obesity (20); having maintained a stable body weight for the past 3 months; lack of use of medications known to affect weight and menstrual function; having a low diabetes risk as assessed by a risk factor questionnaire for diabetes and insulin resistance (21). All subjects provided written consent and the study was approved by the Ethics Committee in the Faculty of Medicine at the University of Alberta. Subjects were recruited through the University of Alberta campus, surrounding community centers, as well as through the local television station.

Materials and Methods

Study Design

Serum leptin response to GnRH, serum LH response to GnRH, 24-hour urinary cortisol, and energy availability were investigated in a repeated measures intervention study extending over approximately 7 months. Obese women received individualized nutrition and exercise counseling to facilitate loss of at least 10% of their body weight.

Before and after subjects lost 10% of their body weight, the following variables were measured in the early follicular phase of the menstrual cycle (defined as being the first 7 days of the menstrual cycle, where day one constitutes the first day of their menstrual period; 22): energy intake (4-day diet records), resting energy expenditure (REE; indirect calorimetry), body fatness (underwater weighing), 24-hour urinary cortisol, serum leptin and LH (serial blood measurements), and leptin pulsatility. These variables were assessed within 4 weeks of the 10% loss of body weight, during which body weight loss was maintained at a loss of 10% of body weight.

Nutrition and exercise counseling was based on analysis of an initial 4-day diet and exercise record, using Food Processor II Nutrient Analysis Software (ESHA Research, Salem, OR.), a food frequency questionnaire (23), and a discussion of previous dietary and activity practices. The ESHA Food Processor II Nutrient Analysis Software calculated energy expenditure through the conversion of metabolic equivalents (defined as a standard quantity of oxygen required per kg of body weight, per minute, under resting conditions; (24)) into calories (25). At the initiation of the study, a 2-hour individual meeting was held with the subjects and the counselor to discuss proposed nutrition and exercise changes. All subjects were encouraged to attend diet counseling sessions weekly throughout the duration of the study. Counseling sessions ranged from 15 minutes to 1 hour. The interviewer began all interviews by asking participants to describe the previous day in terms of food and eating and encouraged participants to elaborate about the challenges they had regarding eating and exercise. Subjects were weighed once a month in the early follicular phase of the menstrual cycle.

Weight and exercise management was accomplished through nutrition and exercise education as well as behaviour therapy and was provided in written and verbal form. Nutrition and exercise recommendations were made in accordance with the Canada Food Guide for Healthy Eating (26) and Canada's Physical Activity Guide (27) respectively. Although diet programs were individualized, general nutrition information was given to the subjects throughout the study and included: decreasing portion sizes, eating a variety of foods, eating less fat/choosing low fat foods, eating healthy fats, eating more fibre, eating healthy snacks (ie low in sugar/fat), drinking 8-12 cups of water each day, avoiding excessive intakes of regular soda (ie not diet), keeping healthy snacks

accessible, and planning meals and snacks. Additionally, to prevent overeating, subjects were encouraged to keep a daily record of food eaten (only if it helped prevent subjects from eating too much), eat regular meals and snacks, eat slowly, drink water with meals, use smaller plate sizes, make a healthy grocery list (which was often checked with the nutrition counselor), and utilize behaviour modification techniques.

Although exercise programs were individualized to subjects needs, all exercise programs were developed for subjects in the same manner. Before the exercise program was initiated and developed, each subject filled out an exercise questionnaire answering questions about desired exercise time, frequency, equipment/program availability, and schedule of time available to exercise. A gradual integration of regular exercise was incorporated for each subject to minimize the amount of change endured by each subject. The exercise program initially focused on exercise frequency versus the amount of time spent exercising, to establish a consistent exercise routine, which involved exercising at least 3 times per week. Once the subject was exercising consistently, exercise goals regarding the amount of time spent exercising were made which involved exercising 30 minutes per day, 3 times per week. Once the exercise routine was established with respect to time and frequency, the idea of intensity was introduced where subjects were encouraged to exercise at a level where they were breathing hard, but still able to carry on a conversation. After the exercise routine was established with respect to time, frequency and intensity, the duration of the study involved setting exercise goals that were based on increasing the time spent exercising and/or increasing the time spent exercising at a more challenging intensity.

Underwater weighing was used to assess the body composition of each subject. The instrumentation consisted of a tank filled with water at a temperature of approximately 35 C and an electronic scale with a carrying capacity of 10 kg and 10-g precision (28), from which a chair frame is suspended. Each subject was immersed, and after maximal expiration while in the seated position, their weight under water was recorded to the nearest 10 g. Six to 10 underwater weighing trials were performed, with the 3 most consistent trials, within 50 grams, averaged and used as the underwater weight. The residual volume was calculated using a regression equation (29). Using the measured dry weight, the measured weight under water, and the RV, the body density of each subject was determined (28). Percent body fat was subsequently determined by substituting the calculated value for body density (BD) into the Siri equation (30).

Prior to the measurement of REE and respiratory exchange ratio (RER), subjects were instructed not to consume any food/beverages 12 hours before the morning testing period, not to engage in any physical activity 24 hours before the testing period, and to lie in a supine position for 30 minutes prior to testing. Measurements were done in a quiet, dimly lit room at a constant temperature. The REE and RER were determined using the Sensormedic VMAX n29 metabolic cart (Summit Technologies, Anaheim, Ca) located in the Nutrition Laboratory, Faculty of Agriculture, Forestry, and Home Economics for 20-30 minutes pre- and post-weight loss. Resting energy expenditure was calculated using the formula of Weir (31).

Menu programs and individualized exercise program (based on 4-day diet records, the food frequency questionnaire, and exercise history) were given to subjects throughout the study to provide subjects guidance about how much to eat, how to modify

their regular eating patterns, and the amount, type, and intensity of exercise to perform. The recommended amount to eat was based on a desired weight loss of 0.5-1.0 kg per week and was calculated by multiplying the measured REE by an activity factor. Activity factors were coded as follows: less than once a week (sedentary), activity factor = 1.3; 1-3 times each week (moderate), activity factor = 1.5; more than three hours each week (active), activity factor = 1.7 (ESHA Research, 1990; 32). When subjects experienced difficulty losing weight, a 4-day diet and activity record was collected and analyzed to give the subject feedback on diet and exercise changes that could be made to the weight loss program. Goals were re-set, and challenges and strategies used to overcome challenges were discussed.

Urine Collection

Urine was collected a minimum of 24 hours after the last bout of exercise or blood measurement, and was collected before the blood collection. The 24-hour urinary cortisol was assessed with a urine collection that began after emptying morning bladders, where subjects collected samples throughout the next 24 hours whenever their normal urinations occurred. The collection of the first void the following morning indicated the completion of the collection. Subjects were instructed to store their urine in a cold place (at 4C) during collection. The urine was delivered to the researcher immediately following the 24-hour collection. The importance of not missing collection times was emphasized to each subject. Two 10 ml well mixed aliquots were saved and frozen at -80 C until analyzed.

Blood Collection

Blood was collected during the first 4-7 days of the menstrual cycle, to assess serum leptin and LH concentrations before and after a 10% loss of body weight. Following an overnight fast and a 12-hour refrain from exercise pre- and post-weight loss, subjects were admitted to a study room where an indwelling intravenous (IV) catheter connected to a 3-way stopcock was inserted between 0700-0900. Two milliliters of blood were collected every 10 min, for 8 hours. Two hours into the blood collection, an IV bolus injection of 100 ug of a GnRH agonist (gonadorelin hydrochloride; Factrel, Wyeth-Ayerst, Montreal, Quebec) was administered. Blood samples remained at room temperature for 45-60 minutes then centrifuged, and serum was aliquoted and stored at -80C until analyses were performed. At 1200 hours, subjects ingested a packed lunch that the subject had prepared. Foods and beverages consumed were recorded.

Radioimmunoassays

Commercially prepared radioimmunoassay (RIAs) kits were used for serum leptin (Linco, St. Charles, MO; 33), serum LH (Incstar, Stillwater, MN; 34), and 24-h urinary cortisol (Incstar, Stillwater, MN; 35, 36) to determine the mean of duplicate sample determination. Samples were analyzed on the same day to minimize interassay variability.

The intra-assay coefficient of variation for the high (12.9-26.8 ng/ml) and low (2-4.2 ng/ml) leptin quality controls was 6.3% and 7.3% respectively. The inter-assay CV for leptin was 5.9%. The intra-assay coefficient of variation for the high (122-168 mIU/ml) and low (17-21 mIU/ml) LH quality controls was 5.3% and 4.8% respectively.

The inter-assay CV for LH was 8.0%. The intra-assay coefficient of variation for the high (28.3-37.5 ug/dL) and low (3.1-4.7 μ g/dL) cortisol quality controls were 4.1% and 8.8% respectively. The inter-assay CV for cortisol was 8.5%. For LH, leptin, and cortisol assay performance, refer to **Appendices I, II, and III** respectively.

Statistical Analysis

For each subject, the area under the curves (calculated as the average concentration for that time period; 37) for the first two hours (defined as the baseline AUC) and hours two to eight of the blood measurement (defined as the post-GnRH bolus AUC) were determined. The following LH variables were used to assess the hypothalamic-pituitary responsiveness to the GnRH bolus: the difference in LH concentration from baseline to peak (calculated as the maximum LH concentration obtained after the GnRH bolus was given minus baseline LH levels; 37), time elapsed to reach the maximum LH concentration after the GnRH bolus was given (37), the rate of LH concentration increase from baseline LH concentration to the maximum LH concentration reached after the GnRH bolus was given, and the rate of LH concentration decrease from peak LH concentration to final LH concentration. Energy availability was calculated by determining the difference between energy intake (assessed with a 4-day diet record) and energy expenditure (assessed with a 4-day exercise record), both obtained pre- and post-weight loss.

Leptin pulse analysis was conducted using cluster analysis with the Munro 1.1. (Zaristow software, Scotland) hormone pulse profile program. Specific settings for the leptin pulse analysis were determined using the method described by Veldhuis and

Johnson (38), which allowed the researcher flexibility in selecting cluster sizes of test peaks and pre- and post-peak nadirs while minimizing the potential for false-positive errors. For this analysis, a cluster configuration of 2 points for the nadir and 2 points for the peak were selected. A *t* statistic of 2.0 was used in this study and has been used in other studies (39) to provide optimal sensitivity (> 90%) and accuracy. For missing data points, the time point was deleted and the data was moved up to fill the gap (Munro 1.1, Zaristow software, Scotland). The following mean properties of pulsatile leptin concentrations were analyzed: pulse frequency (number of significant peaks/8 hours), interpeak interval in minutes, pulse height, nadir, pulse area, and fractional pulse height (percent increase over the nadir; 100% corresponds to the nadir)

To determine the differences between pre- and post-weight loss variables, paired *t*-test were performed, where a significance level was defined at $p = 0.05$. Significant differences between LH and leptin variables were determined using an ANOVA and Tukey's post-hoc test. ($p = 0.05$). Associations between variables were determined through the calculation of the Pearson product moment correlation coefficients and were calculated for: leptin and LH variables; leptin variables and 24-h urinary cortisol; LH variables and 24-h urinary cortisol; leptin variables and energy-related variables; LH variables and energy-related variables; leptin/LH variables and anthropometric variables. All results are presented as the mean $\pm$ SEM. The SAS statistical package (Cary, NC) was used to analyze the data. The minimum number of subjects needed to find significant differences was calculated to be five (40), and was based on previous changes and variability in leptin levels with weight loss (41).

Results

Subject Description

The total sample size recruited from approximately 100 women was 19 Caucasian women. Fourteen out of 19 subjects dropped out of the study after participating in the study for 3.6 ± 0.6 months. Results from subjects who voluntarily decided that they did not want to continue to participate in the study were excluded because these subjects were not successful in losing 10% of their body weight. Participants who successfully lost 10% of their body weight ($n = 5$) required an average of 7.3 ± 0.1 months to do so. By the end of the study, all weight loss subjects were exercising 30-60 minutes/day, 3-5 times per week.

The average age and height of the subjects successful in losing weight was 31.6 ± 1.4 years and 168.7 ± 2.2 cm respectively. Subjects were not restricting energy intake 3 months prior to initiation of the study, but had engaged in previous dieting activity (defined to subjects as restricting energy intake) inconsistently for a period of 3.4 ± 0.7 years. Sixty percent of subjects had lost and regained at least 10 pounds three times or more in the past 5 years. Twenty percent of subjects had tried to lose weight more than three times in the past year. The initial BMI value was 34.9 ± 1.7 kg/m².

Anthropometric, metabolic, and nutritional changes with 10% weight loss

After 10% weight loss, a significant decrease in BMI, % body fat, and fat mass was observed (Table 3.1). Post-weight loss absolute energy availability, energy availability corrected for fat free mass, the absolute value of the REE, and the REE corrected for fat-free mass decreased in comparison to pre-study values (Table 3.2).

Table 3.1: Characteristics of obese, eumenorrheic women before and after 10% loss of body weight* ($\bar{x} \pm \text{SEM}$).

Variable	Pre-study	Post-study
Weight (kg)	$98.6 \pm 3.6^{***a}$	88.2 ± 3.6^b
BMI (kg/m^2)	34.9 ± 1.7^a	30.6 ± 1.5^b
Body fat (%)	42.3 ± 2.7^a	34.1 ± 2.6^b
Fat mass (kg)	42.1 ± 4.1^a	30.2 ± 3.0^b
Fat free mass (kg)	56.6 ± 1.1	57.9 ± 2.5

*10% weight loss required 7.3 ± 0.1 months ($n = 5$).

^{a,b}Significantly different with different letter superscripts, $p < 0.05$.
Comparison made by paired t-tests.

Energy intake (2512 ± 370 kcal/day to 1620 ± 143 kcal/day), protein intake (96.6 ± 12.8 g/day to 68.6 ± 3.4 g/day), carbohydrate intake (322.6 ± 31.9 g/day to 233.4 ± 26.8 g/day), total fat intake (93.2 ± 26.3 g/day to 46.8 ± 4.4 g/day), polyunsaturated fat intake (13.2 ± 3.5 g/day to 8.4 ± 1.4 g/day), monounsaturated fat intake (28.4 ± 13.8 g/day to 13.8 ± 1.1 g/day), saturated fat intake (30.8 ± 7.7 g/day to 14.6 ± 1.8 g/day; $10.4 \pm 1.0\%$ of total energy intake to $8.0 \pm 0.9\%$ of total energy intake), omega-6 fat intake (7.13 ± 0.16 g/day to 3.97 ± 0.87 g/day), and trans fat intake (1.97 ± 0.46 g/day to 0.49 ± 0.20 g/day) were lower after the 10% weight loss period. Omega-3 fat intake (0.67 ± 0.08 g/day to 0.64 ± 0.21 g/day) remained essentially the same after weight loss. All pre- and post-study nutrients requirements were met, except for the post-study protein requirement (42).

Percent calories coming from carbohydrate before ($53.2 \pm 4.9\%$) and after ($56.0 \pm 1.6\%$) weight loss remained in the recommended range of 50-60% (40). Percent calories coming from fat before ($30.6 \pm 4.5\%$) and after ($25.6 \pm 1.2\%$) weight loss was within the recommendation that fat account for no greater than 30% of calories (40). Percent calories coming from protein before ($15.2 \pm 4.9\%$) and after ($16.8 \pm 1.0\%$) weight loss were essentially within or slightly above the recommended range of 10-15%. In accordance with the protein requirements of 0.8 g/kg/day (40), protein intakes pre-study (96.6 ± 12.8 g/d) easily met protein requirements (70 g/d), while protein intakes post-study were slightly under the requirement (68.6 ± 3.4 g/d).

Table 3.2: Energy variables in obese eumenorrheic women before and after 10% weight loss* ($\bar{x} \pm \text{SEM}$).

Energy Variable	Pre-study	Post-study***
Exercise energy expenditure (kcal/day)**	145 ± 40	241 ± 78
Energy intake (kcal/d)**	212 ± 370^a	1620 ± 143^b
REE (kcal/day) ^c	1911 ± 115^a	1692 ± 40^b
(kcal/kg body weight)	19.5 ± 0.8	19.3 ± 0.6
(kcal/kg FFM)	33.9 ± 1.1^a	29.4 ± 1.1^b
RER (VCO_2/VO_2)	0.68 ± 0.01	0.71 ± 0.02

*10% weight loss required 7.3 ± 0.1 months ($n = 5$).

**Assessed using Food Processor II Nutrient Analysis Software (ESHA Research, Salem, OR.)

***Assessed after 10% weight loss.

^{a,b}Significantly different with different letter superscripts, $p < 0.05$.

^cMeasured by formula of Weir (1949).

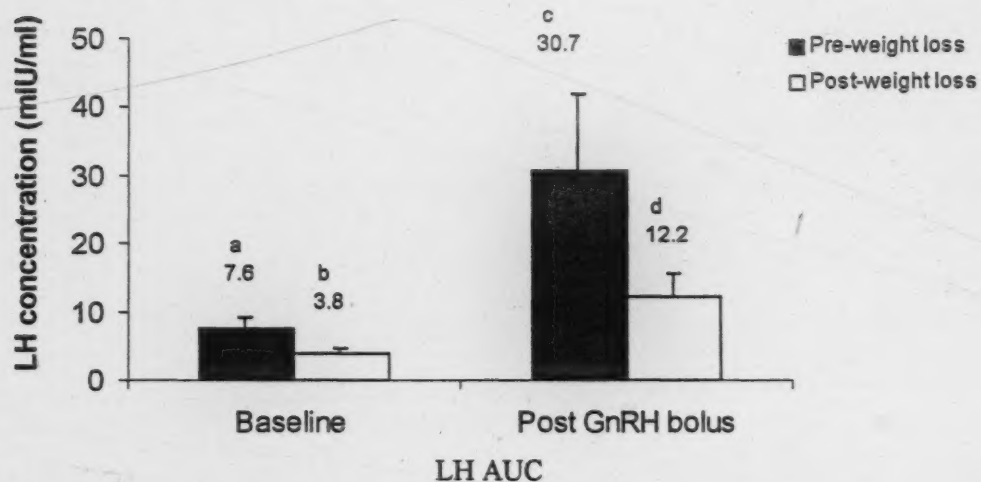
Comparisons made by paired t-tests.

LH AUC and response to GnRH

Following weight loss, baseline LH AUC and post-GnRH bolus LH AUC were smaller in comparison to pre-weight loss values ($p < 0.001$; Figure 3.1). Following weight loss, baseline LH AUC and post-GnRH bolus LH AUC decreased $51.0 \pm 2.1\%$ and $53.1 \pm 8.2\%$ respectively from the pre-weight loss values. All post-weight loss LH variables decreased in comparison to pre-weight loss values when corrected for weight, fat mass, and fat free mass, except for the baseline LH AUC, which was not different pre- and post-weight loss when corrected for these variables.

Before weight loss, post-GnRH bolus LH AUC increased $352.1 \pm 75.2\%$ from the baseline LH AUC. After 10% weight loss, the LH post-GnRH bolus AUC increased $329.0 \pm 22.1\%$ from the baseline LH AUC.

Figure 3.1: Luteinizing hormone (LH) AUC in obese eumenorrheic women before and after weight loss



^{a,b} Significant difference between pre- and post-weight loss baseline LH AUC, $p < 0.001$.
^{c,d} Significant difference between pre- and post-weight loss post-GnRH bolus LH AUC, $p < 0.001$.

^{a,c} Significant difference between pre-weight loss baseline LH AUC and pre-weight loss post-GnRH bolus LH AUC, $p < 0.05$.

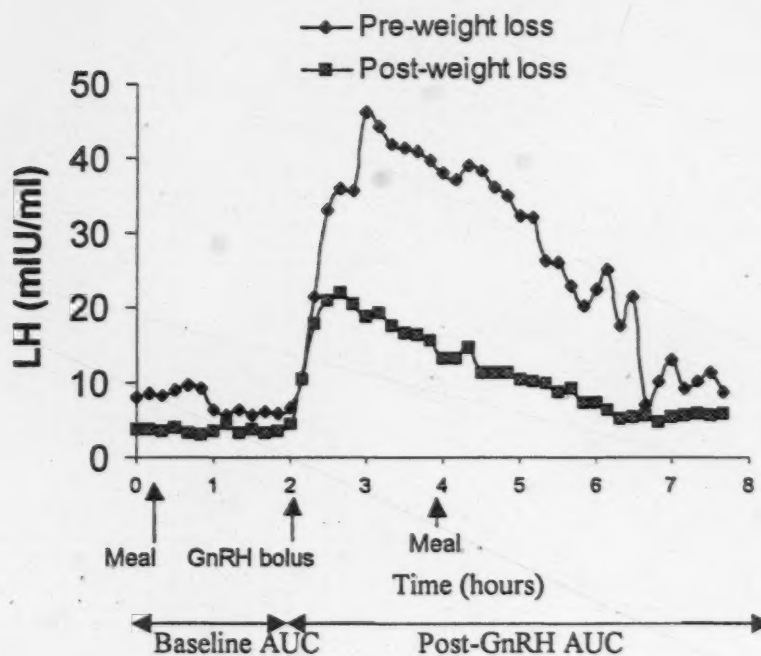
^{b,d} Significant difference between post-weight loss baseline LH AUC and pre-weight loss post-GnRH bolus LH AUC, $p < 0.05$.

Comparisons made by repeated measures ANOVA and Tukey's test.

The serum LH response to the GnRH bolus was diminished following weight loss (Figure 3.2). Post-weight loss peak serum LH concentration (peak [LH]) obtained after the GnRH bolus was given (21.5 ± 4.3 mIU/ml) was lower than the pre-weight loss value (53.8 ± 16.7 mIU/ml; $p < 0.05$). The rate of increase from baseline to peak serum LH concentration (slope increase from baseline (b) to peak (p) LH value; $\Delta [LH]_{b-p} / \Delta \text{time}$)

decreased post-weight loss (0.63 ± 0.19 mIU/ml min) compared to the pre-weight loss value (1.05 ± 0.20 mIU/ml min; $p < 0.05$).

Figure 3.2. Serum LH concentration profile: Blood collected at 10 minute intervals from 0800 to 1600 with the addition of a GnRH bolus (100 ug) at 1000 in the follicular phase of the menstrual cycle in obese ($n=5$) eumenorrheic women before and after loss of 10% of body weight.



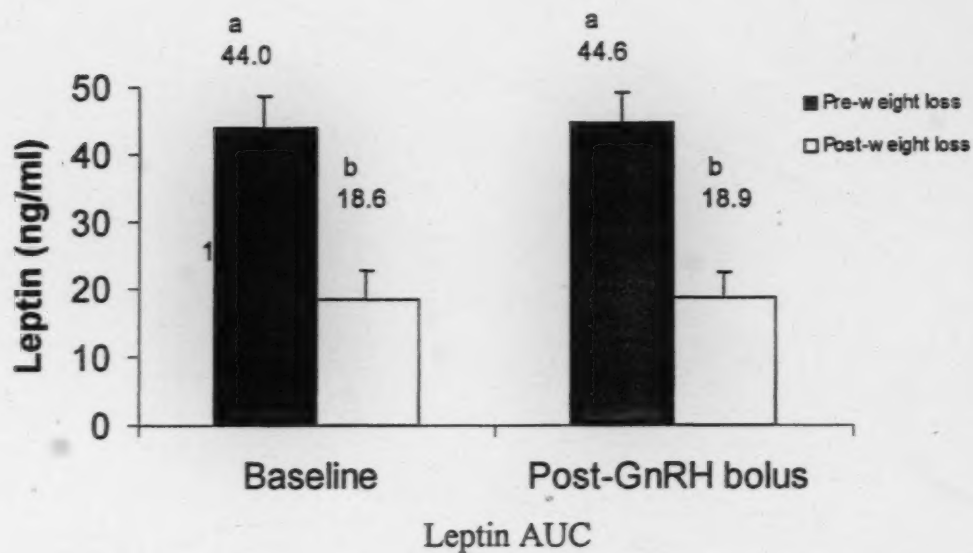
SEM bars removed for clarity

Leptin AUC and pulsatility following weight loss

Baseline and post-GnRH bolus leptin AUCs decreased in comparison to the baseline values ($p < 0.001$; Figures 3.3 and 3.4). Baseline leptin AUC and post-GnRH bolus leptin AUC decreased $58.7 \pm 5.8\%$ and $58.0 \pm 4.7\%$ respectively, in comparison

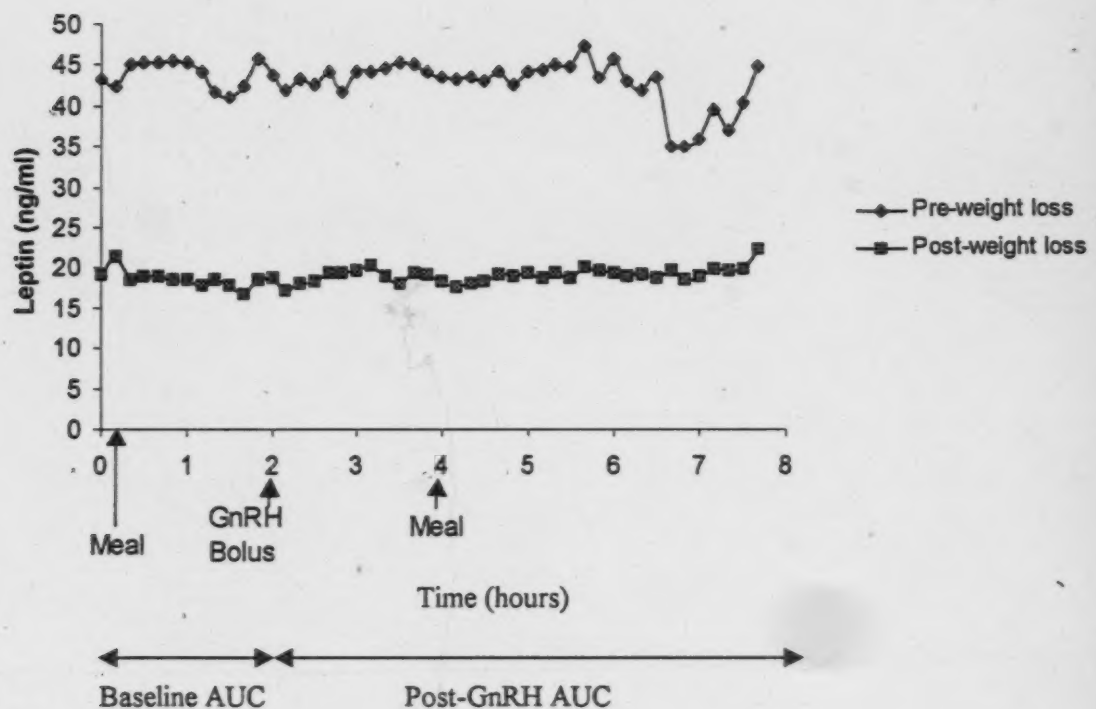
to pre-weight loss values. Baseline leptin AUC/fat mass (0.62 ± 0.12 ng/ml kg) and post-GnRH bolus leptin AUC/fat mass (0.63 ± 0.10 ng/ml kg) was lower compared to the pre-weight loss values (Baseline leptin: 1.08 ± 0.13 ng/ml kg; Post GnRH bolus leptin: 1.10 ± 0.16 ng/ml kg; $p < 0.05$).

Figure 3.3: Leptin AUC in obese eumenorrheic women before and after 10% weight loss.



^{a, b}Significant difference between pre- and post-weight loss baseline leptin AUC, and between pre- and post-weight loss post-GnRH bolus leptin AUC, $p < 0.001$.

Figure 3.4. Serum leptin concentration profile: Blood collected at 10 minute intervals from 0800 to 1600 with the addition of a GnRH bolus (100 ug) at 1000 in the follicular phase of the menstrual cycle in obese (n=5) eumenorrheic women before and after loss of 10% of body weight.



SEM bars removed for clarity

Leptin pulsatility characteristics before and after weight loss changed significantly (Table 3.3). After a 10% loss of initial body weight, leptin pulse height (2.84 ± 0.85 ng/ml) decreased compared to the pre-weight loss value (7.67 ± 1.46 ng/ml; $p < 0.05$). Post-weight loss leptin pulse area (103 ± 31 ng/ml) and leptin nadir (18.1 ± 3.4 ng/ml) decreased compared to the pre-weight loss values (669 ± 128 ng/ml; 39.4 ± 4.4 ng/ml, respectively; $p < 0.001$).

Table 3.3: Leptin pulsatility characteristics in obese, normally menstruating women before and after 10% weight loss (n=5)*.

Parameter	Pre-study	Post-study	Change
Frequency (min^{-1})	1.60 ± 0.81	4.20 ± 1.69	2.60 ± 1.22
Pulse Interval (min)	514 ± 129	281 ± 136	-233 ± 119
Pulse height (ng/ml)	7.67 ± 1.46	2.84 ± 0.85	-4.83 ± 0.79^a
Area (ng/ml)	669 ± 70	103 ± 31	-566 ± 66^b
Nadir (ng/ml)	39.4 ± 4.4	18.1 ± 3.4	-21.2 ± 2.5^b
Fractional pulse height (%)	120 ± 2	116 ± 3	-4 ± 2

*Analyzed using Monroe 1.1, paired t-test.

^aSignificant difference between pre- and post-study value, $p < 0.05$.

^bSignificant difference between pre- and post-study value, $p < 0.001$.

Urinary Cortisol

Urinary cortisol levels before ($54.35 \pm 9.61 \mu\text{g}/24 \text{ h}$) and after 10% weight loss ($55.16 \pm 6.7 \mu\text{g}/24 \text{ h}$) were not significantly different.

Correlations between leptin and LH

No significant correlations were found between pre- and post-study leptin and LH AUCs, as well as between the changes in the leptin and LH AUCs. However, significant relationships existed between leptin and LH response to the GnRH test. The rate of decrease in serum LH concentration from the peak value to the final value obtained over the blood measurement was inversely related to the post-GnRH bolus leptin AUC ($r = -0.89$). The baseline to peak serum LH concentration ($\Delta [LH]_{b-p}$ (mIU/ml) was positively correlated to the baseline leptin AUC ($r = 0.85$).

Correlations between LH, energy-related variables, and anthropometric variables

Luteinizing hormone values correlated with energy-related variables. Pre-weight loss time to peak serum LH concentration was negatively correlated to pre-weight loss energy expenditure ($r = -0.90$; $p < 0.05$) and was positively correlated to pre-weight loss energy intake, and energy availability ($r = 0.93$; $p < 0.05$). Table 3.4 describes significant relationships between change in LH variables and change in energy-related variables.

Significant correlations were not found between LH variables and fat mass or body fat. The change in weight with 10% weight loss was significantly correlated to the changes in baseline LH AUC ($r = 0.92$, $p < 0.05$), post-GnRH bolus LH AUC ($r = 0.95$, $p < 0.05$), the change in time to peak serum LH concentration from baseline ($r = 0.94$, $p < 0.05$), and the change in peak serum LH concentration ($r = 0.91$, $p < 0.05$).

Table 3.4: Significant correlations ($p < 0.05$) between change in LH variables and changes in energy-related variables in obese, normally menstruating women as a result of 10 % weight loss ($n = 5$).

LH variable	Energy variable	Correlation Coefficient
Δ baseline LH (mIU/ml)	Δ Energy expenditure (kcal/day)	0.92
Δ post-GnRH bolus LH (mIU/ml)	Δ Energy intake (kcal/day)	0.91
Δ post-GnRH bolus LH (mIU/ml)	Δ Energy availability (kcal/day)	0.92
Δ [LH] _{b-p} / Δ time [†] (mIU/ml minute)	Δ Energy availability (kcal/day)	-0.87
Δ in time to peak [LH] from baseline (minute)	Δ Energy intake (kcal/day)	0.89
Δ in time to peak [LH] from baseline (minutes)	Δ Energy availability (kcal/day)	0.91
Δ in peak [LH] (mIU/ml)	Δ Energy intake (kcal/day)	0.87
Δ in peak [LH] (mIU/ml)	Δ Energy availability (kcal/day)	0.89

[†]Change in LH concentration from baseline to peak/change in time; slope increase to peak LH value from baseline value.

Correlations between leptin, energy-related variables, and anthropometric variables

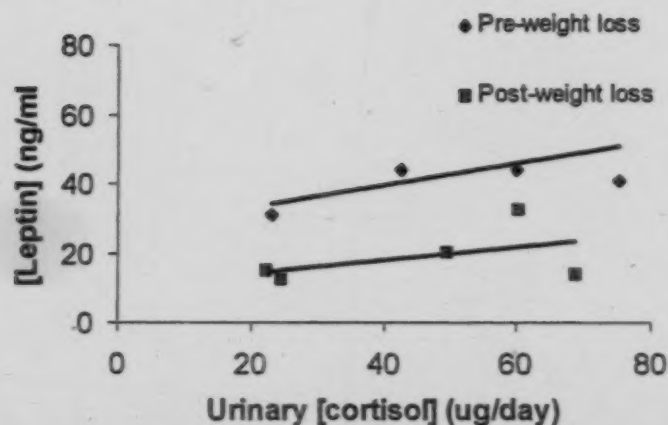
Leptin variables were significantly correlated to energy-related variables. Pre-weight loss REE was positively correlated to pre-weight loss baseline leptin AUC, post-GnRH bolus leptin AUC, leptin pulse amplitude, and leptin nadir ($r = 0.89 - 0.97$). Pre-weight loss leptin pulse area was negatively correlated to the pre-weight loss energy expenditure ($r = -0.92$) and positively correlated to energy intake ($r = 0.90$), and energy availability ($r = 0.91$). Correlations between the post-weight loss REE and baseline leptin AUC ($r = 0.82$, $p = 0.09$), post-GnRH bolus leptin AUC ($r = 0.84$, $p = 0.07$), and leptin nadir ($r = 0.85$, $p = 0.06$) approached significance. Change in leptin pulse frequency

(min^{-1}) was positively related to change in energy expenditure (kcal; $r = 0.95$). There were no significant correlations between leptin variables and anthropometric variables.

Correlations between urinary cortisol, LH, and leptin

There was no correlation between LH variables and urinary cortisol concentration ($\mu\text{g}/24$ hours). No significant correlation between leptin AUCs and urinary cortisol concentration ($\mu\text{g}/24$ hours; Figure 3.5) was observed. However, significant correlations existed between the change in 24-h urinary cortisol and the change in leptin pulse frequency ($r = 0.89$, $p < 0.05$).

Figure 3.5: Regression of plasma leptin concentration (ng/ml) versus urinary cortisol concentration ($\mu\text{g}/\text{day}$) before and after loss of 10% of body weight in obese, eumenorrheic women ($n = 5$).



No significant differences were noted between regression lines generated independently pre- and post-weight loss. Regression coefficients between leptin concentration (ng/ml) and urinary cortisol concentration ($\mu\text{g}/\text{day}$) pre- and post-weight loss were respectively 0.45 and 0.21.

Discussion

In this study we demonstrate a 50% reduction in serum leptin levels as a result of 10% weight loss in obese eumenorrheic women. Lower leptin levels were related to alterations in sensitivity of the hypothalamic-pituitary axis to GnRH following energy restriction and exercise. The leptin-mediated regulation of the reproductive axis were not related to changes in the HPA axis or body fat, but may have been related to changes in energy metabolism. These results may have implications for the use of leptin as a pharmacological agent to treat more severe menstrual disorders.

LH and leptin changes

The decreased LH concentrations occurring with an energy restriction in the current study have been well documented by previous research (43), suggesting that energy restriction reduces GnRH pulse generator activity, thereby resulting in decreased LH levels. In addition, the LH response to a GnRH bolus was similar to other studies utilizing this protocol (44). The suppressed LH response to a GnRH bolus with weight loss is consistent with previous research finding that LH pulsatile parameters improved after recovery of lost weight (45). Increase in serum LH in response to the GnRH bolus made it difficult to correctly assess pulsatility parameters for serum LH.

Leptin levels did not change in response to a GnRH bolus, which is consistent with previous research finding that GnRH release does not influence leptin dynamics (46). Because serum leptin did not change in response to a GnRH bolus, leptin pulsatility parameters were assessed over the 8-hour blood measurement. The $48.4 \pm 7.7\%$ decrease in the average serum leptin concentration (pre-weight loss: 44.3 ± 4.7 ng/ml; post-weight

loss: 18.8 ± 3.7 ng/ml) as a result of 10% weight loss extending over 7 months in obese eumenorrheic women, was consistent with other long-term weight loss studies finding that obese women (whose initial weight, BMI, body fat, and initial serum leptin levels were very similar to the subjects in this study) who lost 9% and 13% of body weight, experienced a 50% (47) and 41% (48) decrease in serum leptin concentration respectively. The results of this study are in contrast to other studies finding smaller decreases in serum leptin with larger decreases in weight loss (41).

Variable decreases in serum leptin with weight loss may be due to differing lengths and severities of the energy restriction, obesity level of subjects, age of subjects, changes in body fat distribution of subjects, and the method of blood collection. High resistance to leptin action in obese subjects may result in a smaller decrease in serum leptin levels with weight loss (48). Smaller decreases in serum leptin with weight loss in previous studies may have also been due to the older age of subjects studied, where leptin production and levels may be decreased with age (49). Discrepancies between studies about the changes in serum leptin with weight loss may have also been due to the variability associated with the change in body fat distribution with weight loss, which was not assessed in the current study. A multiple blood sampling protocol to assess leptin pulsatility, compared to collection of a single blood sample may have also contributed to variable responses to weight loss.

In the current study, serum leptin did not decrease in proportion to decreases in fat mass and is similar to results obtained in other studies (47, 48). The decrease in leptin was larger than the decrease in fat mass. This may have been due to: disproportionate decreases in serum leptin at different stages of weight loss (47, 48); changes in leptin

resistance such that less leptin would be required for the same physiological effect (50); loss of more subcutaneous fat compared to visceral fat, as leptin mRNA levels are substantially greater in subcutaneous than in omental adipocytes, especially in women (48); and loss of more abdominal fat mass, which may increase insulin sensitivity, subsequently resulting in larger decreases in serum leptin levels (50).

Although leptin pulsatility parameters differed from pulsatility parameters in energy replete, normal weight women (2, 50), the pulsatility parameters were similar to those found for obese women (51). Loss of 10% of body weight resulted in significant changes in leptin pulsatility parameters, which included a decrease in pulse height, pulse area, and nadir. The decrease in leptin pulse height, without a change in pulse height in relation to the nadir (51), the decrease in leptin pulse height with decreases in fat mass (52), and the decrease in leptin nadir without significant changes in leptin pulse frequency and pulse duration (51) are consistent with other studies evaluating the effect of an energy restriction on leptin pulsatility. However, the results of the current study are difficult to compare to other studies evaluating the impact of an energy restriction on leptin pulsatility (50) due to differing menstrual phase measurements and differing levels of obesity. Thus, the current study represents the first study where the effect of an energy restriction in an obese population, assessed in the follicular phase of the menstrual cycle, on leptin pulsatility was evaluated.

Leptin and reproductive function:

The relationship between leptin and reproductive function was evaluated by monitoring serum leptin and LH concentrations at baseline and in response to a bolus of

GnRH. Results from this study suggest that changes in serum leptin do not parallel changes in serum LH levels. This is consistent with findings that leptin infusion into male monkeys (53) and pubertal male monkeys (54) did not prevent the changes in serum LH concentrations observed with a short-term energy restriction. Short- and long-term energy restrictions in normal weight women and in infertile obese women reduced serum leptin levels that were unrelated to changes in serum LH (51, 55). These results suggest that leptin could act principally at the ovarian level to affect reproductive endocrine parameters. *In vitro* studies conducted on human thecal and granulosa cells showed that leptin has inhibitory (56) and stimulatory effects (57) on ovarian steroid output. Further studies are needed to clarify if leptin plays a role in communicating the metabolic status to the reproductive system by affecting ovarian hormone production.

Leptin and sensitivity of the reproductive axis

Although there were no significant correlations between serum leptin and LH concentrations, significant correlations existed between the serum leptin and the LH response to a GnRH bolus. These results suggest that leptin may have altered the pulsatile secretion and/or sensitivity of the pituitary to GnRH. This is consistent with Nagatani (58) who found that changes in pituitary responsiveness occurring as a result of decreases in plasma leptin, mediated the decrease in LH pulse frequency with a 48-hour fast in adult female rats. Exogenous leptin prevented the suppression of LH pulse frequency in fasted adult female (59), female mice (10), and male monkeys (14). Leptin enhanced the responsiveness of the anterior pituitary to GnRH stimuli and potentiated GnRH release from hypothalamic explants *in vitro* (13).

Assessment of the relationship between LH frequency and amplitude and leptin concentration/pulsatility characteristics may have provided additional evidence that leptin affected sensitivity of the hypothalamic-pituitary axis. However, this was not possible due to the fact that an artificial pulsatile pattern of LH was created through the addition of the GnRH bolus. To determine specifically that a change in sensitivity of the reproductive axis occurred with an energy restriction as a result of leptin mediation, the study would have to be repeated without the addition of a GnRH bolus. This would allow for a more accurate assessment of the relationship between the actual LH pulse frequency and amplitude and leptin.

Mechanism by which leptin affects sensitivity of the reproductive axis

The lipostatic hypothesis predicts that change in serum leptin concentrations would be directly proportional to change in body content and LH levels with an energy restriction, and that change in serum LH concentrations would be directly proportional to change in body fat. In the current study, this hypothesis is refuted because: no relationship was found between LH variables and body fat content; decreases in serum leptin with an energy restriction did not occur in direct proportion to decreases in body fat; decreases in serum leptin concentrations were not related to decreases in serum LH concentrations, although serum leptin was related to changes in sensitivity of the hypothalamic-pituitary axis of the reproductive axis. Thus, leptin affects the sensitivity of the reproductive axis by another mechanism other than one directly involving body fat content.

It is also possible that leptin affects the sensitivity of the reproductive axis by minimizing the suppression exerted by the HPA axis on release of GnRH and gonadotropins (10). However, in the current study, it is not likely that leptin affects sensitivity of the reproductive axis through the HPA axis because: 1) 24-h urinary cortisol levels did not increase with an energy restriction; 2) there was no relationship between 24-h urinary cortisol concentration and serum leptin concentrations or serum LH concentrations. Although there was a significant correlation between the change in 24-h urinary cortisol and leptin pulse frequency, the lack of increased 24-h urinary cortisol levels with an energy restriction in addition to the lack of a relationship between 24-h urinary cortisol concentration and LH variables suggest that leptin does not affect the sensitivity of the reproductive axis through the HPA axis. Cortisol levels in the current study were within the normal range of 10-100 ug/24 h (Incstar, Stillwater, MN; 35, 36).

Absence of a relationship between leptin and cortisol is consistent with research showing that administration of leptin does not affect elevated cortisol levels occurring as a result of a 42-57 hour fast in male rhesus monkeys (53). Additionally, a 6 month hypocaloric diet (250-350 kcal/day deficit) in 54 obese, postmenopausal women resulted in a 7.1% decrease in body weight, a 14.5% decrease in leptin concentrations, but no change in cortisol concentrations (60). These results are in contrast to other studies, which have found that leptin may blunt the rise in cortisol secretion occurring with an energy restriction (17). Results may differ between studies linking leptin, reproduction function and the HPA axis due to various species studied, blood collection protocols, and energy restrictions utilized.

Cortisol, which is increased during periods of stress (10), may not have increased with weight loss because weight loss may not have changed the stress levels of these subjects. Lack of stress changes with weight loss is supported by research investigating psychosocial weight loss characteristics in this same group of subjects. This research found that these subjects were able to manage challenges associated with weight loss effectively due to large amounts of support from family and friends, and experienced increases in self-confidence as a result of weight loss. Thus, psychosocial data obtained from other research involving these subjects suggests that cortisol levels did not change with weight loss because stress levels did not change with weight loss.

Given the documented effects of metabolic fuel availability on reproduction (4) and the effects of leptin on fuel oxidation (15) and metabolic rate (61), it is also possible that leptin may indirectly affect sensitivity of the reproductive axis through its effects on energy availability. Alternatively, leptin may respond to changes in energy availability, which may then affect the reproductive axis either directly or indirectly through neuropeptides (ie neuropeptide Y; 16). The metabolic model predicts that with an energy restriction: decreases in energy availability will be significantly related to decreases in serum LH and serum leptin; changes in leptin variables will be significantly related to changes in LH variables; decreases in leptin will occur out of proportion to decreases in body fat content. Decreases in LH variables in relation to changes in metabolic variables (seen by correlations between energy-related variables and LH variables with an energy restriction) are consistent with the metabolic model and with previous research showing that changes in LH pulsatility occurred as a result of decreases in energy availability (43).

The metabolic model is also supported by the presence of a significant relationship between leptin and energy-related variables. This supports previous research showing that there is a positive relationship between REE and serum leptin, as leptin has been shown to increase metabolic rate (61). The relationship between leptin and REE with weight loss is controversial and some research has shown no relationship, suggesting that leptin is not the primary signal that mediates the changes of energy expenditure that accompany the maintenance of an altered body weight in humans (62). In the current study, the relationships established between serum leptin and energy-related variables were between pre-weight loss variables, although correlations between post-weight loss variables approached significance. Additionally, weight loss and exercise may have improved the sensitivity to leptin action such that a lower physiological leptin concentration would have been needed to elicit biological effects of leptin.

Results from this study suggest that leptin may influence the sensitivity of the reproductive axis through energetic fuel availability. Previous research supports this by finding that fasting-induced infertility was not reversed by leptin when fatty acid oxidation and glucose utilization were prevented (15). Signals generated by changes in enzymes involved in fat or carbohydrate metabolism might originate in tissues such as brown adipose tissue, skeletal muscle, pancreas, ovary, and pituitary. These signals may then reach the hypothalamic-pituitary-ovarian axis by being modulated by leptin or by modulating the action of leptin. Further research is required to examine relationship between leptin, metabolic variables, and reproductive function.

In conclusion, the present study shows that serum leptin levels and serum LH levels decrease with a 10% loss of weight in obese, eumenorrheic women. Although decreases in serum leptin are associated with changes in sensitivity of the pituitary response to GnRH, a causal link between leptin and sensitivity of the pituitary response to GnRH was not established. The lack of change in 24-h urinary cortisol, as well as the decrease in leptin out of proportion to decreases in body fat suggest that leptin does not affect reproductive endocrine parameters through the HPA axis or through concepts associated with the lipostatic model respectively. Relationship between energy-related variables and leptin/LH variables suggest that leptin may affect reproductive endocrine parameters through a metabolic link. In this case, leptin may facilitate reproductive function based on the requirement of the presence of sufficient metabolic fuels for oxidation. However, leptin may not be the only permissive signal which mediates reproductive endocrine parameters on this basis, as other factors that respond to changes in energy availability have been suggested to be involved (63). This demonstrates the possible limitations of leptin as a treatment for infertility if leptin does in fact affect reproductive function through metabolic effects, where any therapy that increases energy expenditure and inhibits energy intake may be restricted in its capacity to facilitate the energetically costly processes associated with reproduction. Future research should clarify the relationship between leptin and energy metabolism, investigate the mechanism by which leptin and energy metabolism may be linked, and investigate the mechanism by which this link will affect reproductive function. Results from these research areas may provide information about pharmacological methods that can be developed to treat menstrual disorders.

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Chapter 4

Knowledge, history, challenges, and strategies characteristic of weight loss participants, non-completers, and non-responders.

INTRODUCTION

Obesity, "an excess of body fat frequently resulting in a significant impairment of health" (1, p. 157), is a major public health problem. The prevalence of overweight and obesity (body mass index (BMI) $> 25 \text{ kg/m}^2$) among Canadian adults in 2001 was approximately 50% (2). Dieting is one of several approaches to combat obesity, yet despite the billions of dollars spent on weight reduction programs, their success is questionable (3).

Given the large number of people trying to lose weight, the characteristics of successful and unsuccessful short- and long-term outcomes remains an important goal to optimize program results. Teixeira et al (4) observed successful dieters to have a positive self-concept in contrast to unsuccessful dieters, whose negative self-concept was characterized by unrealistic expectations for weight loss, greater body dissatisfaction, low self-efficacy, and a higher number of recent dieting attempts. A higher perceived stress from one's weight may manifest itself through the creation of unrealistic goals (4). Body dissatisfaction is associated with binge eating behavior (5); which is associated with increased drop out rates (6) and excessive exercise tendencies (7) in weight loss participants. Additionally, low levels of self-efficacy, defined as the confidence in the ability to exercise or decrease portion sizes in the context of different emotional states or situations (8), is associated with low levels of weight loss (9) and weight maintenance (10, 11).

Experiencing stressful life events can make weight loss efforts more difficult. A greater percentage of weight loss participants who had lost weight, but regained it, reported the occurrence of major illnesses, bereavements, and other environmental stressors at the end of treatments as compared to weight loss participants who were able to maintain their weight losses (12). Other researchers suggest that it is not stress that determines success of the weight loss participant, but rather the ability to cope with stress (8). Various studies show that weight loss participants (13, 14) and weight loss maintainers (15, 16) are able to generate coping responses to more situations and use effective problem solving to cope with stress. Rather than managing problems directly, unsuccessful weight loss participants report using "escape-avoidance" ways of coping such as eating, sleeping more, or wishing the problem would go away (17). This is in contrast to successful dieters who are more likely to attribute problem solving to strategies (such as meal plan) that were more personally controllable, and to confronting behaviors, like calorie balancing skills (18). Thus, enhanced problem solving skills may characterize a successful weight loss participant.

In a weight loss program, adequate support can facilitate the management of difficult situations, such as gender-role conflicts (which involve childcare and household duties), body dissatisfaction, and lack of time. This was suggested by the observation that spousal, social, and familial support improved diet and exercise adherence and enhance weight lost (19, 20). The basic premise behind support is that family and friends, if cognizant of appropriate eating/exercise habits and skills, may model, suggest, support, reinforce positive behaviors for the individual trying to lose weight (20), help weight loss participants learn greater self-acceptance, develop new norms for

interpersonal relationships, and help manage stressful work or family-related situations (21). For example, spousal and family support with childcare and household responsibilities, especially for women in the work force, can facilitate regular exercise (22). Support may override the guilt associated with committing time to one's own needs, versus the needs of the family. Thus, support not only facilitates weight loss management by overcoming gender role conformity (the idea that a woman has a sense of responsibility to other than to self; 11), but also provides time to engage in weight management behaviors, as time has been cited as a the main barrier to weight loss (23, 24). Thus, support structures offer great potential for the overweight and obese women who face considerable barriers to weight loss behaviors.

Successful dieters are also characterized by being regularly active (25). Although a number of studies evaluating the effects of weight loss with diet versus diet plus exercise have found that weight loss attributable to increased physical activity is small (26, 27), physical activity has been shown to be one of the key correlates of sustained weight loss (28-30). This may be due to improvements in psychological functioning that result from exercise (for example, improved body image, psychological well-being, and eating self-efficacy), which may help obese people better cope with and be less reactive to stressful situations and reduce overeating and the resultant negative emotions that lead to relapses (28, 31).

Based on the multiple, interacting factors identified to affect weight loss success, Social Cognitive Theory (SCT) may provide a model from which to investigate weight loss success as it explains behavior in terms of a triadic, dynamic, and reciprocal model in which behavior, personal factors, and environmental influences all interact (32). The

continuing interaction among the characteristics of a person, the behavior of that person, and the environment within which the behavior is performed is called reciprocal determinism (32). Social cognitive theory also incorporates how self-efficacy affects behavior changes, which has been suggested to have a role in determining weight loss success (33).

Although the studies evaluating characteristics of successful and unsuccessful dieters are limited by the variable definitions of what a successful dieter is (34), distinct differences between successful and unsuccessful dieters have been documented. Recently, two groups of unsuccessful dieters have been identified which include participants who do not complete the program and participants who may complete the program, but are unable to lose weight (4). Previous characterization of these two groups of dieters unable to lose weight has not been documented. Thus, the purpose of this study was to explore and describe the perceptions and experiences influencing eating and exercise patterns of participants able to lose weight, participants who do not complete (non-completers) the program, and participants who complete the program but are unable to lose weight (non-responders). Concepts from SCT were used to provide a theoretical basis for understanding the experiences of weight loss participants. Understanding the practices of dieters who successfully lose weight, who aren't able to complete the program, or who aren't able to lose weight may allow for a better matching of interventions to participants, saving resources, and increasing program efficacy.

METHODS

Design This research used an exploratory descriptive design with qualitative methods of data collection and analysis. The counseling sessions were part of an intervention study that evaluated various metabolic variables in response to weight loss. Because women in the metabolic study experienced difficulty losing weight, the analysis of narrative data collected from counseling sessions previously conducted in a different study, was initiated in an effort to understand the challenges encountered and the strategies attempted throughout a weight loss program.

Qualitative methods were used to elicit weight loss participants' experiences articulated in their own words within the context of their personal environment. Documentation of participants' actual experiences during counseling sessions provided immediate feedback on weight loss interventions and participants' personal experiences including unanticipated influences on success. These factors have all been previously cited as valid reasons for use of qualitative research designs (35, 36).

Participants. Healthy, regularly menstruating, non-smoking, obese, Caucasian women were recruited for a long-term weight loss program. Inclusion criteria were: being sedentary for the past year (exercised less than 20 minutes each week); having a BMI between 30-40 kg/m², which was indicative of obesity (37); having maintained a stable body weight for the past 3 months; lack of use of medications known to affect weight and menstrual function; and having low diabetes risk, as assessed by a risk factor questionnaire for diabetes and insulin resistance (38). All subjects gave informed written consent and the study was approved by a University Ethics Committee. Participants were

recruited using posted notices describing the study's objectives and the benefits it would provide for the participants and through "health watch segments" at the local television news station. Notices were posted around the University campus, surrounding community centers, and fitness facilities.

Counseling Sessions. History of eating/exercise practices, and weight management efforts attempted were obtained in the initial interview and through demographic questionnaires about previous dieting and activity. To document dietary intake and energy expenditure, a 4-day diet and exercise record was collected before and after weight loss which were statistically evaluated using the SAS statistical package (Cary, NC). The diet and exercise record was evaluated using Food Processor II Nutrient Analysis Software (ESHA Research, Salem, OR.). Loss of 10% of body weight was encouraged through weight loss education and guidance, as well as behavior modification techniques. Weight loss management consisted of a comprehensive 2-hour counseling session, followed by weekly 15-60 minute counseling sessions throughout the 7-month study. These counseling sessions were conducted at locations convenient for participants. One person (SV) conducted all sessions and the interviewer was trained as an exercise leader and nutritionist. Participants were not counseled during weeks that they were ill or on vacation.

In the initial interview and subsequent counseling sessions, participants' beliefs about their diets were intentionally discussed within the context of recent food- and exercise-related experiences. The interviewer avoided direct questions about diet and exercise guidelines and compliance so that participants did not feel that they were being

tested (39), and encouraged participants to describe in their own words those beliefs that they had used to shape and understand their recent food-and exercise-related situations. Questions and responses were relayed to the participants in a neutral, non-judgmental, and interested manner.

The initial interview involved a great deal of nutrition and exercise education and served as the basis for nutrition and exercise intervention. This meeting was based on the analysis of the initial 4-day diet record and covered information related to nutrition education (ie weight management, portion size, Canada's Food Guide, fiber, fat etc), past dieting and exercise practices, behaviors associated with eating (ie emotional eating), shopping, meal/menu planning, healthy recipes, and instruction on how to evaluate their own diet.

Weekly counseling sessions encompassed the following topics: prior 24-hour eating and exercise diary; discussion of short-term and long-term eating and exercise challenges; development of eating and exercise strategies, monitoring progress of goals, and providing feedback; discussion of high risk eating and exercise situations. The topics were addressed through a list of semi-structured questions that were asked at every counseling session (except for questions regarding future challenges, which were asked if time was available; Table 4.1).

Although counseling sessions did not directly incorporate SCT, they did incorporate the impact of personal and environmental factors on weight loss behaviour. Throughout the study, the participant and facilitator attempted to set realistic and manageable goals for exercise adherence and weight loss. Contingency contracts (that

did not involve food) were developed for the accomplishment of goals (20). If participants experienced difficulty with an

Table 4.1: List of semi-structured questions asked during weekly counseling sessions.

<i>Topic</i>	<i>Question</i>
24-h recall	What foods have you eaten in the last 24 hours? What exercise have you done in the last 24 hours?
Challenges	What eating/exercise challenges (if any) did you experience this week? How do you feel that your eating exercise routine went this week?
Strategies	What strategies did you use to overcome challenging situations? If you encountered a similar challenging situation again, what strategy would you take to overcome it?
Problem-solving	Do you foresee any challenging eating/exercise situations in the coming week? If so, how do you plan to manage it?

eating/exercise situation, stimulus control procedures were used to change the antecedents of the behavior (20). Environmental cues and behavioral chains were identified and managed by enlisting stress management techniques, time management principles, and support systems. To attain goals and to assist in managing a 'high-risk' period, menu plans were provided at the request of the participant. All participants received exercise programs and the option of having an exercise companion. As the study progressed, the topics covered in the interviews remained the same, but became more specific as participants became more familiar with their own issues.

Data collection

All counseling sessions were reconstructed from detailed notes taken by the counselor during and after counseling sessions, in accordance with participants' perspective on all aspects of the counseling program described. Data collection was discontinued when subjects dropped out of the study or when subjects lost 10% of their original body weight.

Coding and analyses.

Baseline demographic variables with numerical data were summarized with descriptive statistics. Differences in baseline variables between groups were assessed using analysis of variance and tukey's post-hoc test (SAS Statistical Package, Cary, NC, 2001).

The qualitative coding process involved reviewing interview field notes a number of times to classify discrete incidents, ideas, or events (ie concepts) in the data. The coding categories that developed from the interviews were established by previous knowledge of factors affecting weight loss (4, 8, 19, 22, 28), as well as recurring issues and themes that arose spontaneously from the data. Data suggestive of similar meanings were coded according to the following classifications: past eating and exercise patterns, challenges to weight loss, strategies for weight loss, and mediating factors involved in weight loss. These data were further classified into time sequence categories which included: study initiation; the first 3 months of the study; and the last 3 (to 4) months of the study.

Following descriptive coding, analysis involved content analysis that classified similar events, people, processes, or meanings that develop naturally from the interviews according to broad conceptual themes rather than concentrating on quantification (40). To get a more thorough view of feelings and ideas of participants, thematic content analysis was used to create categories of similar persons and processes occurring over time, which materialized from the interview sessions. Thematic content analysis is the categorization of verbal or behavioural data with the intent of finding a theme in the content (41).

Various methods were used to enhance trustworthiness (42). One researcher (SV) coded and analyzed the data and the second investigator (KR) reviewed the data and coding frameworks. Peer debriefing (43) involved obtaining feedback from researchers competent in qualitative research, but not involved in the study. Trustworthiness was also enhanced through the use of low inference descriptors (44) that were very close to the participants' accounts and researchers' field notes. A partial member-check (45) was employed where participants successful in losing weight were asked for feedback on the researcher's interpretations and conclusions. Triangulation was used to enhance trustworthiness and involved the use of field notes and interview notes to verify results (45). Recall bias and bias occurring as a result of prolonged engagement with participants were minimized by having a defined, specific, and consistent (data collection was neutral, non-judgmental) data collection process, and by having a registered dietitian sit in on and interpret results of some participant interviews to ensure consistency and accuracy.

RESULTS AND DISCUSSION

Participant characteristics. For the 19 subjects recruited, the mean BMI value was $33.8 \pm 1.5 \text{ kg/m}^2$, and the mean age was 30.6 ± 1.6 years. The participants all graduated from high school, and 7/19 were college graduates. Half of the participants were married, some with children, either working full/part time (Table 4.2).

Fourteen out of 19 participants did not lose 10% of their body weight after participating for 3.64 ± 0.58 months (designated minimal weight loss group). Of these 14 participants, 8 participants dropped out of the study after 2.13 ± 0.35 months (designated non-completers). This represents an attrition rate of 42%, which is within the attrition rate range of 23-64% for clinical-based weight loss programs (46-49), and slightly lower than the attrition rate of 50-95% found for commercially-based programs much higher than the average attrition rate for weight loss programs of about 20% in the last 10 years (50). The large variability in attrition rates for weight loss programs may be due to differences in weight loss programs evaluated, the subjects studied, and the environmental context surrounding weight loss (i.e. if there was financial reimbursement; if there was a large health risk etc).

A group of 6 participants not able to lose weight remained in the study 5.67 ± 0.61 months ($p < 0.001$) significantly longer than non-completers (designated non-responders). Non-responders can also be considered to have participated in the weight loss program for as long as the participants in the weight loss group, as the duration of involvement for these two groups of participants did not differ. Participants who successfully lost 10% of their body weight ($n = 5$; designated weight loss group) participated for an average of 7.3 ± 0.12 months.

Thus, 2 types of participants in the current study were not able to lose 10% of their body weight: non-completers and non-responders. This is consistent with previous research investigating attrition and weight loss where non-completers and the least successful completers represented 2 different groups who did not lose weight (4).

Prior eating behavior, exercise patterns, and weight control efforts. As a group, the non-completers and non-responders reported a longer history of past dieting activity (7.9 ± 1.5 years vs. weight loss group: 3.4 ± 1.7 years, $p < 0.05$). Breaking this down even further, non-responders reported a longer history of past dieting activity (7.2 ± 1.0 year) as compared to the weight loss group (3.4 ± 1.7 years, $p < 0.05$). In contrast, non-completer's previous time spent dieting (8.5 ± 2.6 years) was not significantly different from that in the weight loss group, primarily due to the large variability in past dieting practices for the non-completers. Four of 8 non-completers, and 3/6 non-responders reported more frequent past dieting activity when compared to frequent past dieting activity reported by 1 participant in the weight loss group ($n = 5$; $p < 0.01$).

Greater past dieting activity may have made it more difficult for the minimal weight loss group to lose weight as others have reported that a high previous frequency of dieting predicted failure of weight maintenance after a

Table 4.2: Characteristics of weight loss group participants, the minimal weight loss group*, non-completers, and non-responders.

Characteristic ^a	All participants (n = 19) ^β	Weight loss group(n = 5)	Minimal weight loss group ^γ (n = 14)	Non-completers (n = 8)	Non-responders (n = 6)
Age (years)	30.6 ± 1.6	31.6 ± 1.4	29.4 ± 1.3	28.3 ± 1.6	31.0 ± 2.0
Body mass index (kg/m ²)	33.8 ± 1.5	34.8 ± 1.7	32.5 ± 0.9	31.8 ± 1.0	33.3 ± 1.5
Time in study (months)	4.7 ± 0.6 ^{a, d}	7.3 ± 0.1 ^b	3.6 ± 0.6 ^{a, c}	2.1 ± 0.4 ^c	5.7 ± 0.6 ^d
# of counseling sessions attended during study	15 ± 4 ^a	28 ± 1 ^b	10 ± 3 ^a	5 ± 1 ^c	18 ± 4 ^a
Past dieting activity (years)	6.8 ± 1.6 ^{a, b}	3.4 ± 1.8 ^b	7.9 ± 2.1 ^a	8.5 ± 2.6 ^{a, b}	7.2 ± 1.0 ^a
Married	10	2	8	5	3
Children	6	2	4	1	3
Job status:					
Part-time	6	2	4	2	2
Full-time	8	2	6	3	3
Full time mother ^δ	1	1	0	0	0
Mother and works	5	1	4	1	3
Full time student	7	1	6	4	2
Education level:					
Some college	7	1	6	3	3
College graduate	7	2	5	3	2
Annual household income					
less than \$30,000	6	1	5	3	2
\$30,000-\$50,000	6	2	4	2	2
greater than \$50,000	7	2	5	3	2

*The minimal weight loss group includes non-completers and non-responders.

^a Unless otherwise indicated, the unit of measurement is the number of participants.

^β All participants includes the weight loss group and the minimal weight loss group

^γ The minimal weight loss group includes short-term and non-responders.

^{a, b, c, d} Significantly different with different letter superscripts, p < 0.05.

^δ Stay-at-home mom: doesn't work part-time

Comparisons made by analysis of variance and tukey's post-hoc test.

weight loss program (51). Participants mentioned few characteristics that affected past exercise practices, likely due to the lack of regular activity by most participants in the past. One of 19 participants had been a varsity athlete at university, and only two participants had been regularly active in the past.

Key themes from participant narratives.

Temporal changes Three central components of time were identified from the data which described changes in weight loss behaviors occurring over time. At the initiation of the study, all participants described challenges to eating and exercise changes. In the first 3 months of the study, weight loss group participants and non-responders adopted strategies in an attempt to facilitate weight loss, while non-completers struggled with creating strategies for making eating and exercise changes. As a result, non-completers abandoned their weight loss efforts. In the last 3-4 months of the study, environmental and contextual factors, such as problem solving ability, self-efficacy, and support, tested the implementation of weight loss strategies by participants in the weight loss group and non-responders. As a result of these contextual factors, non-responders did not respond to weight loss strategies, while participants in the weight loss group successfully lost 10% of their body weight.

Initial challenges to weight loss management. At the initiation of the study, all participants described similar challenges to eating and exercise changes. Through statements made by participant, participants perceived challenges as those conditions and

behaviors that hindered the formation and preservation of successful lifestyle self-management practices. These perceptions are similar to those described previously (52).

Menu planning was a challenge for 4/5 participants in the weight loss group, 6/8 non-completers, and 4/6 non-responders. The practice of eating out frequently as being part of work culture and a large amount of job changes experienced by 5/6 non-completers and the high frequency of eating out experienced by all 6 non-responders may have contributed to the lack of regular eating and meal planning for these groups.

Exercise challenges All participants cited various barriers to regular activity at the initiation of the study. Four of 5 participants in the weight loss group, 7/8 non-completers, and 4/6 non-responders stated that lack of time was their largest barrier to regular activity: "If I had more time, I could exercise more." This is consistent with previous research stating that lack of time is the most common reason to drop out of an exercise program (22, 31).

Because of their body size, 3 non-completers and 2 non-responders felt unable to participate in sports typical of lean people - "I am too big to exercise" - or due to their feeling of awkwardness exercising in a public place- "When I go to the gym, I feel like people are looking at me and wondering why I am there". Two of 5 subjects in the weight loss group, 5/8 non-completers, and 3/6 non-responders may have avoided exercise and sports settings because of their own feelings of body dissatisfaction: "I really don't like the way I look, and I feel even more self-conscious about how I look when I am exercising with a bunch of fit looking people." This is consistent with previous results reporting that the way obese people perceive their bodies has been shown to be a

determinant of physical activity in a weight loss program (53). Body dissatisfaction as a barrier to exercise was greater for non-completers and non-responders as compared to the weight loss group.

Additional challenges experienced by all study participants for not being able to exercise regularly included family/household responsibilities. Two of the 5 participants in the weight loss group, 2/8 non-completers, and 3/6 non-responders found it difficult to exercise due to family and household responsibilities: "It's hard to exercise when I am always trying to make everybody else happy". This is consistent with gender-role conformity that involves a socialization pattern that emphasizes 'other-directedness' where a woman has a sense of responsibility to other than to self (11). Women with numerous responsibilities such as career, family, and household duties may experience a great deal of guilt when exercising, which in turn, can lead to inactivity (11): "I feel guilty leaving the kids (to exercise) when I have been working all day."

Three of 5 subjects in the weight loss group, 6/8 non-completers, and 3/6 non-responders were not motivated to exercise as indicated by comments like "I just don't enjoy it!" or "I am getting sick of doing the same old thing". Lack of exercise enjoyment and exercise boredom experienced by the minimal weight loss group may have contributed to their inconsistent exercise routine as it has been shown that motivational factors play a critical role in the long-term success of a physical activity-diet modification intervention (54).

In summary, it was expected that time constraints, body size/dissatisfaction, household/childcare responsibilities, and low motivation were barriers to regular activity, as previous literature has cited these factors as challenges to weight loss and exercise.

Body size/dissatisfaction was a barrier that was specific to short- and non-responders, which may have been one factor that contributed to the difficulty associated with trying to lose weight.

However, the novel finding in the current study was that establishing a regular exercise routine presented a far greater challenge to weight loss than eating changes: "I am familiar with changing eating habits, but am not sure how to start exercising because I have not done it before!". Lack of involvement in regular exercise may have made it more difficult for participants to integrate a foreign activity, such as regular exercise, into their life routine.

The first 3 months: the demise of non-completers To overcome weight loss barriers, weight loss group participants and non-responders adopted strategies in an attempt to facilitate weight loss, while non-completers struggled with creating strategies for making eating and exercise changes. The formation and implementation of manageable and progressive goals by participants in the weight loss group and long term attendants facilitated the success of these participants in adhering to their weight loss and exercise programs.

All of the participants in the weight loss group and 4/6 of the non-responders created, implemented, and achieved short-term, manageable, and progressive exercise goals to accommodate their moderate expectations for weight loss: "I know that I can keep it off if I just lose it slowly." These results are consistent with previous research findings that long-term weight loss is enhanced with the development of realistic weight loss goals involving a reasonable weight loss (55).

Realistic goals were characterized by exercise behavior, rather than by a desired outcome and included behaviors such as exercising more frequently, or training for a 5 km running race. These participants increased activity gradually in order to accomplish goals: "I never would have thought that I could run 5 km 6 months ago! I just took it one step at a time." Setting realistic, progressive exercise goals increased exercise adherence for participants in the weight loss group and non-responders because these participants were able to gradually integrate exercise routines into their lives. Thus, an abrupt increase in exercise duration/intensity (which may have been experienced by non-completers) did not become a deterrent to short- and long-term exercise adherence as noted by others (56).

In contrast, 5/8 non-completers were more interested in seeing weight loss changes immediately: "I would like to lose 10 pounds in about 2 weeks." As a result, these participants developed unrealistic goals to reflect the desire for immediate results. For example, 3/8 non-completers drastically restricted energy intake (i.e. ate only one meal a day for 3-5 days), and 3/8 non-completers exercised excessively (i.e. 2-3 hours one day of the week) for a short time frame and expected to see results: "I don't know why I haven't lost any weight this week. I ran for 60 minutes, did weights for 30 minutes, and then rode the stationary bike for 30 minutes." The need for immediacy may have been related to their feelings of body dissatisfaction: "I have GOT to lose weight so that I can look good for my wedding." It is possible that feelings of body dissatisfaction may have driven their need for immediate changes in weight and the engagement in extreme dieting behaviors, such as a severe energy restriction and excessive exercise.

The need for immediacy may have also stemmed from prior frustration associated with previous weight loss efforts: "I have tried so many times to lose weight, I have just got to do it this time." This idea is consistent with previous research suggesting that unrealistic expectations related to the ease, speed, likely degree of change, and presumed advantages associated with making changes may overpower knowledge of one's prior failures (57).

To cope with their inability to achieve weight loss goals, 5/8 non-completers also engaged in avoidance behaviors: "I know that I was supposed to meet you to exercise, but I just had too much to do that day". Six of 8 non-completers were more likely than non-responders and participants in the weight loss group to make excuses and attribute their inability to change to factors out of their control: "I had to work", or "I couldn't exercise because I hurt my foot a year ago." As the study progressed, 6/8 non-completers (compared to 1/6 non-responder and 0 weight loss group participants) attributed their inability to make changes as a result of lack of motivation to exercise as indicated by comments such as "I just don't enjoy it!" or "I am getting sick of doing the same old thing". These results show that as the study progressed, participants in the weight loss group and non-responders overcame the initial barrier of lack of motivation to exercise, while the majority of non-completers were not able to do this. Lack of motivation (58) and avoidance behaviors (18) have been noted to be associated with early drop-outs and may have contributed to this group's inability to lose weight.

After 2 months, non-completers were losing significantly less weight (weight loss = 0.3 ± 2 kg) before they dropped out of the study as compared to weight loss group participants (weight loss = 3.2 ± 0.9 kg) and the non-responders (weight loss = 1.5 ± 0.8

kg). This suggests that the distinguishing barriers for (which include body dissatisfaction and lack of motivation) and strategies (which include unrealistic goals and avoidance behaviors) used by non-completers may have predisposed these participants for non-completion. In summary, when the goals of the non-completers goals were not attained due to of lack of motivation, creation of unrealistic goals, and engagement in avoidance behaviors, the non-completers abandoned their weight loss efforts.

The second 3 months: demise of non-responders At this point in the weight loss program, only participants in the weight loss group and non-responders remained in the study. Thus, the remainder of the discussion will refer only to the participants in the weight loss group and non-responders.

Implementation and achievement of realistic and attainable goals by participants in the weight loss group and non-responders were affected by time management skills, self-efficacy development, amount of support, and problem solving skills. These variables indirectly influenced participants' willingness or ability to acquire or maintain new eating and exercise behaviors.

Time management skills Time management skills had a large impact on weight loss success due to the observation that lack of time was cited as the largest challenge to making exercise and eating changes by participants in the study. All weight loss participants and 4/6 non-responders made eating and exercise changes a priority and made time for these changes as summed up by the following comment: "I know that if I don't make time for it, this program isn't going to work for me". For all weight loss

participants and 4/6 non-responders, integration of exercise into their lives was facilitated by an exercise plan that allotted for an individual and realistic amount of time to exercise: "But just exercising 20 minutes a day doesn't seem so unreasonable and I have no problem fitting the activity into my lifestyle." An exercise duration that was manageable for participants in the weight loss group and non-responders allowed these participants to focus on exercise consistency, rather than amount of exercise performed each day. This is consistent with research showing that frequency of exercise was the strongest predictor of weight loss maintenance (59). A consistent exercise pattern allowed the weight loss group to integrate an exercise program into their lives with greater ease (60).

An increase in daily living activities in 4 of 5 participants in the weight loss group and 2 of 6 non-responders resulted in an increase in overall daily activity and integration of physical activity into the lives of these participants. This was done by exercising during breaks/lunch, taking the stairs instead of the elevator whenever possible, parking farther away from a store so that they had to walk further, increasing activity with household chores, commuting to work/school by foot or bike, and/or incorporating active family activities into their lifestyle. Increases in habitual physical activity in daily life may increase exercise adherence for weight loss participants (22). This is not surprising since an increase in moderate activities outside of planned activities is characteristic of successful weight loss participants (61).

Self-efficacy Self-efficacy issues in this study were related primarily to the participants' confidence in their ability to exercise. Regular exercise increased the confidence and self-worth of all participants in the weight loss group and 3/6 non-responders: "Even

though I haven't lost a ton of weight, I just feel better by exercising." These results are consistent with the observation that regular exercise has a significant impact on those psychological variables most closely associated with the physical changes related to exercise such as changes in perceived fitness and satisfaction with physical shape and weight (9). Confidence in physical ability increased as a result of the exercise program: "Months ago, I never thought I could run 5 km (but I can!)." All participants in the weight loss group and 3/6 of the non-responders experienced a shift from motives such as appearance and weight loss to more intrinsic factors such as mental well-being, sense of fun and achievement, and stress relief as reasons to exercise: "I love dancing around in my apartment - it relieves the stress from my day."

For all participants in the weight loss group, the ability to achieve eating and exercise goals, as well as deal with challenging eating and exercise situations, were facilitated by the increased confidence associated with achieving previous eating/exercise goals and managing previous difficult situations successfully: "I know that I can bike to work because I have been biking that long on the stationary bike" or "I was secure that I could handle it because I had done it before". This is consistent with the notion that a high self-efficacy or self-confidence level about a goal facilitates the accomplishment of that goal (32). The increased confidence levels associated with managing difficult situations or accomplishing a goal provided positive reinforcement for the weight loss group so that they would attempt to manage other difficult situations or accomplish other goals.

This is in contrast to the non-responders who generally did not experience an increase in self-efficacy as the weight loss program progressed, but experienced a

progressive decrease in self-efficacy. Non-responders became very frustrated with their own inability to accomplish a pre-determined goal: "I just don't get it. I am exercising lots and watching what I eat. Why can't I lose weight?" Due to successive failures, non-responders progressively lacked more and more confidence in their ability to accomplish the goal: "Why should I even exercise - it doesn't seem to be doing any good." For non-responders, the decrease in self-efficacy associated with not accomplishing a goal further perpetuated the inability to accomplish future goals and lose weight.

Support. The previous finding that support from spouses was instrumental in determining if eating and exercise changes were adopted into the life routine of a weight loss participant (62) was consistent with the current study. Both participants in the weight loss group who were married received support from spouses for eating differently, and exercising (through assistance with childcare and household duties): "My husband watches the kids so that I can exercise."

This is in contrast to non-responders where the refusal of 2 non-responders spouses (3 non-responders were married) to eat differently (evident by the preparation of two different meals or accommodating the spouse's food preferences, and the lack of assistance with household obligations like childcare and household duties) perpetuated the difficulties associated with attempting to exercise regularly: "I run out of time in my day to exercise because (after work) I have to supervise homework and prepare meals." Thus, the non-responders did not receive as much spousal support as the participants in the weight loss group for making eating and exercise changes. Additionally, non-responders lacked support from other sources such as other family members, or friends

due to distance and lack of time to facilitate meaningful relationships with other people. Lack of support may have contributed to the inability of non-responders to manage difficult situations, as previous research has shown that successful management of weight loss challenges can be facilitated by social support (63).

For 4/5 of the participants in the weight loss group, support during activity may have contributed to promoting exercise regularity, as it has previously been shown that exercising with an exercise partner increases exercise adherence, especially in women (22): "Exercising with Ann every week motivates me to do it." Making a regular commitment to exercise with a friend may have contributed to better time management skills, greater exercise enjoyment, greater socialization, and greater exercise motivation, as was consistent with other women exercising with an exercise partner (22).

Problem solving ability. Problem solving skills affected how the participant was able to deal with high-risk situations, unpredictable situations, and relapse. Problem-solving ability is the mediating variable that differentiated the weight loss group from non-responders. All of the participants in the weight loss group were able to generate coping responses, which is consistent with previous research reporting that weight loss participants who generated coping responses to more situations subsequently lost more weight (15).

Five of 6 non-responders may have experienced greater difficulty overcoming high-risk situations due to the greater magnitude and more difficult nature of their stressors (i.e. job changes, financial problems) as compared to those experienced by the weight loss group (i.e. buffet dinners, lack of exercise equipment). This is consistent

with the previous research finding that major life events such as injury, job changes, and marital breakdown decrease physical activity behavior (64).

Non-responders may not only have had a greater amount of stress in their lives, but may also have had a decreased ability to manage the stress, as the ability to cope with stress is associated with successful weight maintenance and loss (15-17, 28). The non-responders became immobilized by their feelings of guilt and failure, rather than lack of effort and time associated with trying to manage a difficult situation: "It's almost like the guilt I have after missing a workout is more of a barrier than the exercise itself." The overly negative appraisal of their transgression, which may have been a result of decreases in self-efficacy occurring with successive failures in managing challenging situations, may have contributed to the non-responders inability to manage future high-risk situations (65). As a result, non-responders became complacent in attempting to reach their goals: "I guess that this is the way it is. Maybe this is just the size that I am meant to be." That is, they didn't abandon the weight loss program, but stopped trying to accomplish goals, and gradually stopped attending counseling sessions. The inability to manage difficult eating and exercise situations may have been perpetuated by low levels of support available to these participants, and low levels of self-efficacy occurring with the progression of the weight loss program.

Even when the stressor was of equivalent severity (ie missing a workout), the participants in the weight loss group appeared to cope with the stress better than the non-responders. For example, 4/5 participants in the weight loss group expressed feelings of guilt when they did not accomplish a goal, however, they did not let these feelings of guilt immobilize them. Consistent with previous research (66), the weight loss group

learned to be flexible, adjust goals, and learned not to let the guilt overwhelm or halt their progress: "There are some days that I can't fit it (exercise) in when I want to. I just find another time or another day." The ability of the weight loss group to manage difficult situations was further facilitated by increases in self-efficacy occurring with successful management of previous challenging situations, and increases in the amount of support from spouse and family to deal with challenging situations.

CONCLUSIONS

An overview of challenges, strategies and facilitators applicable to each study group is schematically depicted in Figure 4.2. The current study identified characteristics of successful weight loss participants and two distinct groups of participants who were unable to lose weight. Participants who did not complete the weight loss program and dropped out early on appeared to have unrealistic goals, excessive eating and exercise tendencies, and a need for immediate gratification.

Participants who successfully lost weight and participants who completed the program but were unable to lose weight (after expending a great deal of energy and time) were relatively similar. Both groups appeared ready and willing to make a long-term change in lifestyle as was evident by the creation and implementation of realistic goals for eating and exercise challenges. Both groups also appeared to have adequate time management skills to allow them to integrate changes into their lives. Participants successful in weight loss were able to manage difficult situations and attain goals using their time management skills, their problem-solving skills, and by having support to deal with difficult situations and incorporate exercise into their lives.

Although non-responders possessed adequate time management skills, they experienced difficulty managing challenging eating/exercise situations, as well as attaining goals. This was due to decreases in self-efficacy that occurred with successive failures in managing difficult situations and not accomplishing goals. Additionally, the nature of life stressors was much greater than that found for participants in the weight loss group, where the non-responders lacked support and resources to deal with situations which were too challenging for them to handle.

In the current study, adoption of a consistent exercise routine as compared to changing eating habits appeared to have a larger impact on the success of weight loss. Although weight loss was largely due to decreases in dietary intake (calorie intake decreased from 2512 ± 370 kcal/d to 1620 ± 143 kcal/d after weight loss, $p < 0.05$; change in calorie intake was 892 ± 158 kcal/d) rather than increases in energy expenditure (EE; EE increased from 145 ± 40 kcal/d to 241 ± 78 kcal/d insignificantly), increases in SE associated with a consistent exercise routine may have facilitated successful weight loss behaviors such as a problem solving ability. This is consistent with research showing that eating behaviors did not predict weight loss (4) and that addition of exercise to a weight loss program resulted in increases in restraint and decreases in hunger (33). Exercise inclusion in the current study may have facilitated weight loss through the contribution of exercise to total energy expenditure, the role of exercise in facilitating the organization of weight management programs, as well as the decreased anxiety/depression and improved mood/self-esteem seen with a consistent exercise routine (67).

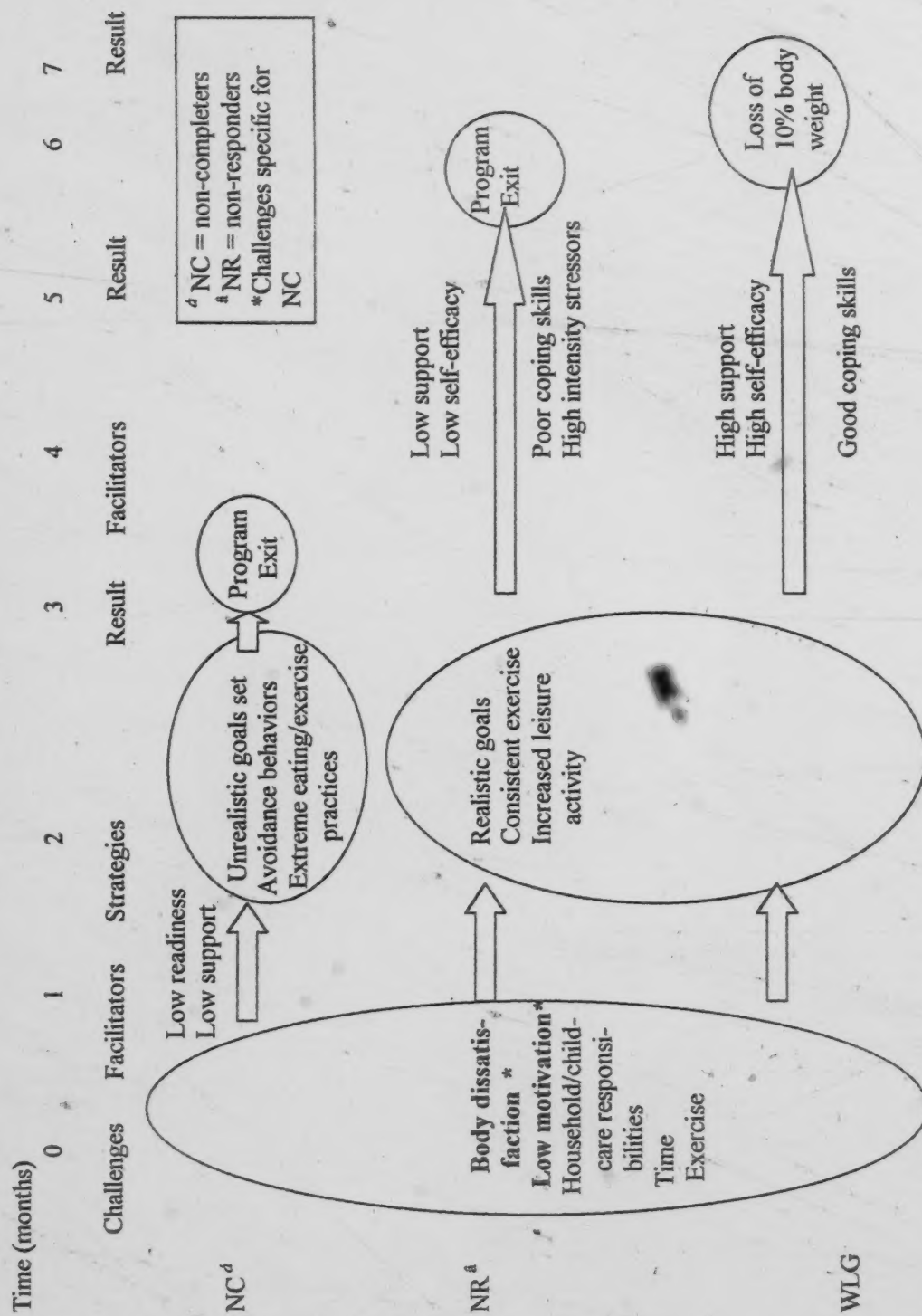
Among study participants, factors and mediating variables were identified that facilitated/impeded eating and exercise patterns influencing daily management effort. Social Cognitive Theory provides a framework for future research to understand the elements that influence health behaviors and suggests intervention strategies to influence future behavior.

Study limitations The limitations of this study need to be considered when evaluating the results. Inadequate representations of participants' views and ideas on weight loss

and exercise may have occurred due to collection of data from a post-hoc study without a clear theoretical base, collection of data from counseling sessions instead of a formal interview, incomplete scope of questioning during counseling sessions, and/or lack of comfort of the subjects to answer the questions honestly.

Limitations in data collection may have also occurred as a result of the fewer number of interviews conducted with short- and non-responders. Attendance of fewer counseling sessions by short- and non-responders may have provided a less accurate representation of short- and non-responders due to the limited amount of data collected. This is in contrast to participants in the weight loss group where a more accurate characterization of this group was possible because they attended more counseling sessions allowing for greater data collection. In addition, the weight loss group was the only group with which data interpretations were checked.

Figure 4.2. Elements influencing eating and exercise patterns among obese women who successfully lost weight (WLG), did not complete the program (NC), or completed the program but did not lose weight (NR).



IMPLICATIONS FOR RESEARCH AND PRACTICE

These findings emphasize the importance of setting realistic goals and reassuring weight loss participants that weight loss takes time and patience. Negative self-concept markers, such as unrealistic goals, body dissatisfaction, and lack of motivation, may indicate that the participant is not ready to make weight changes. Although not studied here, assessment of the stage of change of the participant would allow for a weight loss program that accommodates the participant's level of readiness to change (68).

These findings also emphasize the importance of enabling weight loss participants to manage challenge situations effectively. A multidisciplinary approach to weight that incorporates emotional and spiritual needs would allow weight loss participants better able to cope with life stressors and focus more on the attempted changes. To assist weight loss participants in coping with difficult situations, they should be given direction on effective stress management, identification and management of stressors, problem solving techniques and decision-making skills, and identification and recruitment of appropriate support systems to manage challenging situations. Results from this study suggest that adoption of a regular exercise program should be promoted in a weight loss program to optimize weight loss by: first emphasizing exercise consistency, to establish the exercise habit, then duration, intensity, and frequency; making exercise goals that are progressive and realistic with respect to duration; and by increasing leisure activity.

Future research should involve the development of appropriate tools or procedures to evaluate stage of change of participants who drop out of a weight loss program versus participants who are unable to lose weight, as well as to assess effects of application of the stages of change model on planning and implementation of weight-management programs. Additional factors related to weight maintenance should be investigated to determine if there are different types of weight loss regainers. This may provide information on how to optimize weight maintenance.

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Chapter 5

Summary and Conclusions

1.a. Review of Hypotheses for Study #1

Various factors affect weight loss success. In turn, weight loss, may affect reproductive endocrine blood parameters. The hypotheses tested by this research were:

1. A decreased energy availability in eumenorrheic obese women results in suppressed reproductive endocrine blood markers and decreased leptin levels.
2. Leptin mediates changes in the reproductive axis when energy availability is decreased, through cortisol.

1.b. Review of Study Objectives for Study #2:

i. To determine if there were three types of participants in the weight loss program described in hypotheses #1 and #2 (those who lose weight, those who do not complete the weight loss program, and those who complete the program, but are unable to lose weight) through the analysis of diet counseling data already collected from the weight loss program described in hypotheses #1 and #2.

ii. To explore and describe challenges, strategies and facilitating factors influencing eating and exercise patterns of participants able to lose weight, participants who do not complete the weight loss program, and participants who complete the weight loss program, but are unable to lose weight, through the

analysis of diet counseling data already collected from the weight loss program described in hypotheses #1 and #2.

2. Hypotheses #1 and #2

Hypothesis #1 was verified (Chapter 3) by demonstrating that decreases in weight, induced by decreases in energy availability, in eumenorrheic obese women result in suppressed reproductive endocrine function and decreased leptin levels. Reproductive endocrine blood parameters were suppressed as evident by decreases in LH AUC as a result of weight loss, as well as a decreased response to the GnRH bolus after weight loss. The decreased LH concentrations occurring with an energy restriction in the current study have been well documented by previous research (1), suggesting that energy restriction reduces GnRH pulse generator activity, thereby resulting in decreased LH levels. In addition, the LH response to a GnRH bolus was similar to other studies utilizing this protocol (2). The suppressed LH response to a GnRH bolus with weight loss is consistent with previous research finding that LH pulsatile parameters improved after recovery of lost weight (3).

Hypothesis #1 was supported by a $48.4 \pm 7.7\%$ decrease in post-weight loss leptin from pre-weight loss values, which is consistent (4, 5) and in contrast (6-8) to other studies investigating the effect of weight loss on leptin levels. Loss of 10% of body weight resulted in significant changes in leptin pulsatility parameters, which included a decrease in pulse height, pulse area, and nadir. The decrease in leptin pulse height, without a change in pulse height in relation to the

nadir (9), the decrease in leptin pulse height with decreases in fat mass (10), and the decrease in leptin nadir without significant changes in leptin pulse frequency and pulse duration (9) are consistent with other studies evaluating the effect of an energy restriction on leptin pulsatility. Variable decreases in serum leptin between studies with weight loss may be due to differing lengths and severities of the energy restriction, obesity level of subjects, age of subjects, changes in body fat distribution of subjects, differing menstrual phase measurements, and the method of blood collection. In the current study, these variables were relatively well controlled through the recruitment of women who had similar study characteristics.

Hypothesis #1 is not supported by the lack of significant correlations between serum leptin and LH concentrations. However, Hypothesis #1 is supported by the existence of significant correlations between serum leptin and the LH response to a GnRH bolus. Although the link between serum leptin and the LH response to a GnRH bolus is not causal, these correlations suggest that there may be a relationship between leptin and the response of LH to GnRH. This is consistent with results showing that changes in pituitary responsiveness occurring as a result of decreases in plasma leptin, mediated the decrease in LH pulse frequency with a 48-hour fast in adult female rats and that leptin enhanced the responsiveness of the anterior pituitary to GnRH stimuli and potentiated GnRH release from hypothalamic explants *in vitro* (11).

Hypothesis #2 was not verified by the results of the study because 24-h urinary cortisol levels did not increase with an energy restriction, possibly due to

low levels of stress endured by these subjects. Additionally, there was no relationship between 24-h cortisol concentration and serum leptin or serum LH concentrations. It is possible that leptin may indirectly affect the sensitivity of the reproductive axis through the affect of leptin on energy availability.

Alternatively, leptin may respond to changes in energy availability, which may then affect the reproductive axis either directly or indirectly through neuropeptides (ie neuropeptide Y; 12). This model is supported by: significant correlations between decreases in energy availability and decreases in serum LH and serum leptin; significant correlations between leptin variables and LH variables; significant decreases in leptin out of proportion to decreases in body fat content. These results are consistent with previous research showing that fasting-induced infertility was not reversed by leptin when fatty acid oxidation and glucose utilization were prevented (13). Thus, leptin may influence the sensitivity of the reproductive axis through energetic fuel availability.

2.1. Study #1 Implications and Future Research

Results from the first study suggest that leptin may not have a large role in affecting reproductive endocrine parameters. However, leptin may have a role in affecting reproductive endocrine parameters based on the requirement of the presence of sufficient metabolic fuels for oxidation. If this is the case, then leptin is not the only permissive signal which mediates reproductive endocrine parameters on this basis, as other factors that respond to changes in energy availability have been suggested to be involved (14). This demonstrates possible

limitations associated with using leptin as a treatment for infertility, where any therapy that increases energy expenditure and inhibits energy intake may be restricted in its capacity to facilitate the energetically costly processes associated with reproduction. Although this research suggests that leptin may not be able to be used as a pharmacological agent to treat menstrual disorders, this research provides information about the possible mediators involved in suppressed reproductive endocrine blood markers occurring with an energy restriction. Future research should clarify the relationship between leptin and energy metabolism, investigate the mechanism by which leptin and energy metabolism may be linked, and investigate the mechanism by which this link will affect reproductive function.

2.2. Study #1 Limitations:

Results from the first study are limited by the small sample size. A larger sample size would have provided a more confident representation of the relationship between leptin and reproductive function in obese eumenorrheic women. Additionally, the correlational nature of the data makes it very difficult to determine causal relationships between different variables in the study. Missed blood measurements in the assessment of hypothalamic-pituitary responsiveness may have given a less accurate representation of hypothalamic-pituitary responsiveness.

3. Research objectives and implications for study #2:

Consistent with previous research (15), the study results reveal that 3 groups of participants were found in this weight loss study: participants who lost 10% of their body weight; participants who did not complete the study and did not lose weight (non-completers); and participants who completed the study, but did not lose weight (non-responders).

Study results show that each group of weight loss participants experienced different challenges, implemented different strategies to facilitate change, and had different mediating factors that affected how strategies were implemented. Initial weight loss challenges were similar between all groups except that non-completers and non-responders expressed greater body dissatisfaction than other groups. Non-completers engaged in strategies that were not conducive to weight loss such as setting unrealistic goals, involvement in excessive eating and exercise tendencies, and needing immediate gratification. These participants characteristics are consistent with a weight loss participant who has a negative self-concept (15), and is more likely to drop out of a weight loss program (16).

These findings on non-completers emphasize the importance of setting realistic goals and reassuring weight loss participants that weight loss takes time and patience. Negative self-concept markers, such as unrealistic goals, body dissatisfaction, and lack of motivation, may indicate that the participant is not ready to make weight changes.

Non-responders and successful weight loss participants set realistic goals and managed time well. However, non-responders experienced difficulty managing challenging situations due to large amounts of stress, decreases in self-efficacy and decreased support available to manage the stress. These results are consistent with results showing that high stress (17), decreased self-efficacy (18), poor problem-solving ability (19), and low levels of support (20) can make weight loss efforts more difficult.

These findings emphasize the importance of enabling weight loss participants to manage challenge situations effectively. A multidisciplinary approach to weight that incorporates emotional and spiritual needs would allow weight loss participants better able to cope with life stressors and focus more on the attempted changes. To assist weight loss participants in coping with difficult situations, they should be given direction on effective stress management, identification and management of stressors, problem solving techniques and decision-making skills, and identification and recruitment of appropriate support systems to manage challenging situations.

Although weight loss was largely due to decreases in calorie intake, weight loss behaviors were facilitated by a consistent exercise routine. This is consistent with results showing that exercise inclusion in a weight loss program facilitated weight loss (21-23). Therefore, to optimize weight loss, adoption of a regular exercise program should be promoted in a weight loss program. This can be done by: first emphasizing exercise consistency, to establish the exercise habit,

then duration, intensity, and frequency; making exercise goals that are progressive and realistic with respect to duration; and by increasing leisure activity.

3.1. Study #2 Future Research:

Future research should involve the development of appropriate tools or procedures to evaluate stage of change of participants who drop out of a weight loss program versus participants who are unable to lose weight, as well as to assess effects of application of the stages of change model on planning and implementation of weight-management programs. Additionally, factors related to weight maintenance should be investigated to determine if there are different types of weight loss regainers. This may provide information on how to optimize weight maintenance.

3.2. Study #2 limitations:

The limitations of this study need to be considered when evaluating the results. Inadequate representations of participants' views and ideas on weight loss and exercise may have occurred due to collection of data from a post-hoc study without a clear theoretical base, collection of data from counseling sessions instead of a formal interview, incomplete scope of questioning during counseling sessions, and/or lack of comfort of the subjects to answer the questions completely.

Limitations in data collection may have also occurred as a result of the fewer number of interviews conducted with short- and non-responders.

Attendance of fewer counseling sessions by short- and non-responders may have provided a less accurate representation of short- and non-responders due to the limited amount of data collected. This is in contrast to participants in the weight loss group where a more accurate characterization of this group was possible because they attended more counseling sessions allowing for greater data collection. In addition, the weight loss group was the only group with which data interpretations were checked.

4. Conclusion

Study #1 found that decreases in weight, induced by decreases in energy availability, in eumenorrheic obese women result in suppressed reproductive endocrine blood parameters and decreased leptin levels. Results suggest that leptin does not have a large role in affecting reproductive endocrine parameters, but may some role in affecting reproductive endocrine parameters by influencing the response of LH to GnRH, potentially through the link that leptin has with energetic variables. Study #1 also revealed the difficulty associated with weight loss as was evident by the non-completion of the weight loss program by 8/19 subjects, and the inability of 6/19 subjects to lose weight. Analysis of challenges, barriers, and facilitating factors involved in the weight loss program of non-completers, non-responders, and successful weight loss participants (all performed in Study #2) revealed that non-completers are more likely to have unrealistic goals, body dissatisfaction, and low motivation, while non-responders appear to experience difficulty managing stress and problem-solving. Although

there are limitations associated with the results of the reproductive weight loss study and the study investigating social aspects of weight loss, the results from study #1 has implications for the manipulation of leptin levels for treatment of menstrual disorders associated with an energy deficit, while results from study #2 has implications for clinicians to optimize weight loss.

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Appendices

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Appendix I: Assay performance for LH

LUTEINIZING HORMONE ASSAY PERFORMANCE

Due to the large number of serum samples analyzed for LH, low and high level quality control samples (QC) were run with the standard curve at the end of the assay on each of the 6 testing days. The reported ranges for the QCs were 17-21 mIU/ml for the low level and 122-168 mIU/ml for the high level (DPC®, CON6®). Figures 2 and 3 show the graph of the LH concentration for each singlet of the duplicate sample for each day. From these figures, it is shown that the low and high QCs fell within the expected ranges on both testing days. The mean coefficients of variation (CV) for the duplicate QC values over all testing days were 4.8% for the low QC values and 5.3 % for the high QC values.

Figure 1: Low level quality control concentrations on all LH assay days

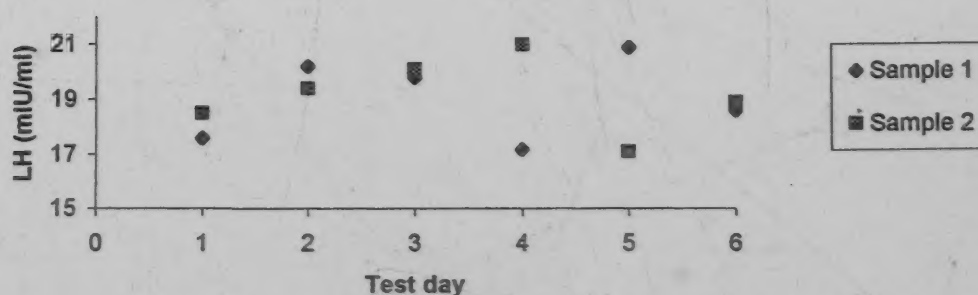
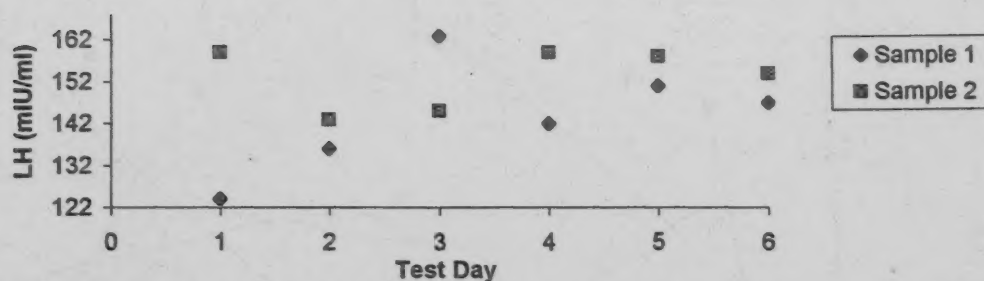


Figure 2: High level quality control concentrations on all LH assay days



The daily intra-assay CV was calculated by averaging the % CV column on the assay output for all of the samples. This calculation did not include standards or QC samples as well as samples with high CVs (which were re-analyzed on the last testing day). Averaging the daily intra-assay CV's (Table 1) resulted in an inter-assay CV of 8.0% for LH.

Table 1: Daily intra-assay coefficients of variation for LH.

Test Day	Pre-study LH %CV	Post-study LH %CV	Average LH % CV
1	11.0	11.4	11.2
2	7.1	8.1	7.6
3	4.1	4.7	4.4
4	7.8	8.4	8.1
5	9.6	11.4	10.5
6	5.8	6.4	6.1
Average	7.6	8.4	8.0

Appendix II: Assay performance for leptin

LEPTIN ASSAY PERFORMANCE

Due to the large number of serum samples analyzed for leptin, low and high level quality control samples (QC) were run with the standard curve at the end of the assay on each of the 6 testing days. The reported ranges for the QCs were 2-4.2 ng/ml for the low level and 12.9-26.8 ng/ml for the high level (Linco, Hornby, Ont.). Figures 3 and 4 show the graph of the leptin concentration for each singlet of the duplicate quality control sample for each day. From these figures, it is shown that the low and high QCs fell within the expected ranges on both testing days. The mean coefficients of variation (CV) for the duplicate QC values over all testing days were 7.3 % for the low QC values and 6.3 % for the high QC values.

Figure 3: Low level quality control concentrations on all leptin assay days

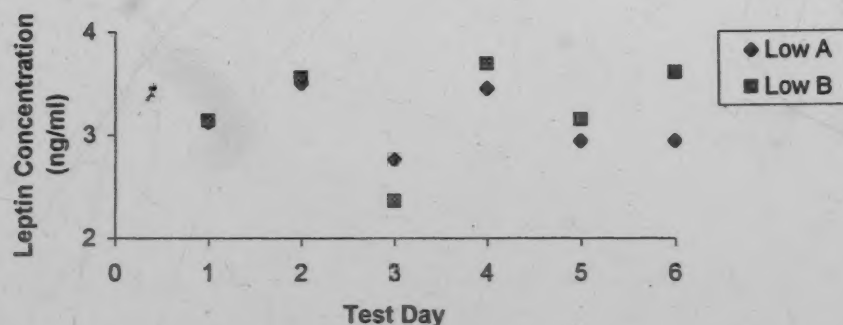
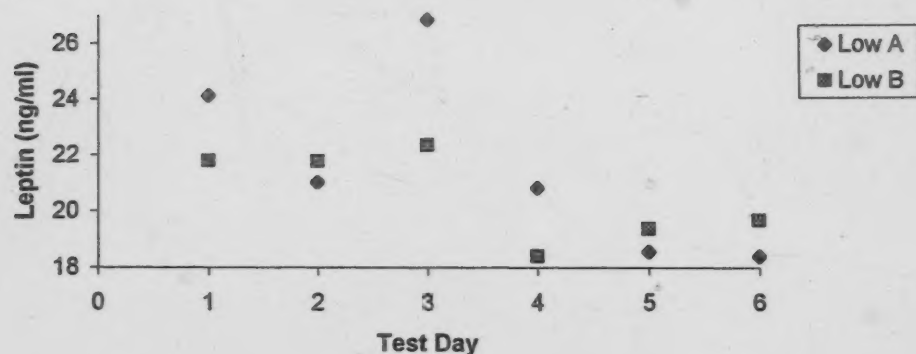


Figure 4: High level quality control concentrations on all leptin assay days



The daily intra-assay CV (Table 2), was calculated by averaging the % CV column on the assay output for all of the samples. This calculation did not include standards, QC samples, or samples with high CVs (which were re-analyzed on the last testing day). Averaging the daily intra-assay CV's resulted in an inter-assay CV of 5.9 % for leptin.

Table 2: Daily intra-assay coefficients of variation for leptin.

Test Day	Pre-study %CV	Post-study %CV	Average pre- and post-study % CV
1	4.3	6.7	5.5
2	6.3	6.9	6.6
3	5.5	6.7	6.1
4	6.1	4.7	5.4
5	6.9	4.7	5.8
6	5.8	5.9	5.9
Average	5.8	5.9	5.9

Appendix III: Assay performance for cortisol

CORTISOL ASSAY PERFORMANCE

Low and high level quality control (QC) values were run with each standard curve on both days of cortisol assays. The reported ranges were 3.1-4.7 ug/dL for the low level control and 28.3-37.5 ug/dL for the high level control (DPC®, CON6®). Figures 5 and 6 show the graph of the cortisol concentration for each singlet of the duplicate quality control sample for each day. From these figures, it is shown that the low and high QCs fell within the expected ranges on both testing days. The mean coefficients of variation for the duplicate QC values on both days were 8.8% for the low QC values, and 4.1% for the high QC values.

Figure 5: Low level quality control concentrations for both cortisol assay days

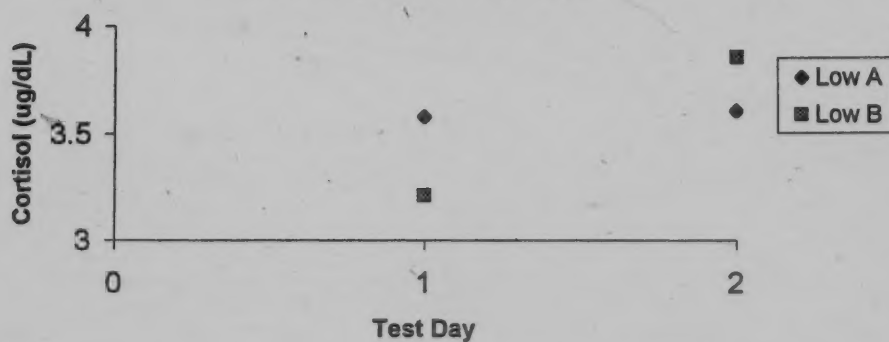
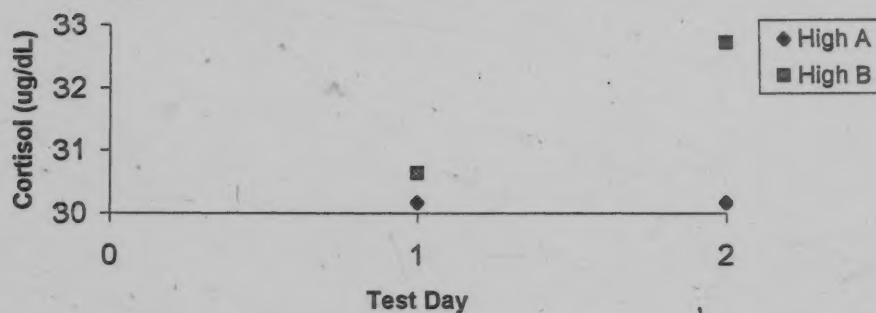


Figure 6: High level quality control concentrations for both cortisol assay days



Intra- and inter-assay performances were assessed. The daily intra-assay CV (7.4% on Day 1 and 9.5% on Day 2) was calculated by averaging the % CV column on the assay output for all of the samples. This calculation did not include standards, QC samples, or samples with high CVs (which were re-analyzed on the last testing day). Averaging the daily intra-assay CV's resulted in an inter-assay CV of 8.5 % for cortisol.